Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Data

Computational insights of novel benzenesulfonamide-1,3,4-thiadiazole hybrids as VEGFR-2 inhibitor: Design, synthesis and anticancer evaluation with molecular dynamics studies

Samir Bondock*[,a], Tallah Albarqi[a] , Moaz M. Abdou[b], Nada M. Mohamed[c]

[a]Chemistry Department, Faculty of Science, King Khalid University, 9004 Abha, Saudi Arabia

[b]Egyptian Petroleum Research Institute, Nasr City, 11727, Cairo, Egypt

[c]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Modern University for Technology and Information, MTI, Cairo, Egypt.

***Corresponding author:** Samir Bondock (Email: bondock@kku.edu.sa, bondock@mans.edu.eg)

1. Materials and instrumentation

1) All melting points were determined on Stuart SMP11 apparatus and were uncorrected.
2) The IR spectra were recorded in KBr discs, on a Jasco FT/IR-460 plus spectrophotometer at College of Science, King Khalid University.
3) The ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 at 850 MHZ on a BrukerAvanceAV-850 NMR Ultrshield^{TM} spectrometer at King Abdulaziz University, Jeddah, Saudi Arabia.
4) Mass spectra were measured using the Shimadzu GC/MS-QP 1000 EX mass spectrometer at 70 eV, at the Micro Analytical Center, Cairo University, Giza, Egypt.
5) Elemental analyses were carried out at the Micro Analytical Center, Cairo University, Giza, Egypt.
6) Biological activities testing was carried out at the Holding Company for Biological Products and Vaccines, VACSERA, Giza, Egypt.

Supplementary S1. Molecular dynamic simulation:

NAMD 3.0.0 employing CHARMM-36 forcefield was used during MD simulation. Protein system construction and preparation were performed following the reported procedures utilizing the QwikMD component within VDW software pertained to reconstructing missing hydrogens under *p*H=7.4 conditions, followed by removing existing co-crystalized water molecules. Following that procedure involved putting the protein structure within an orthorhombic enclosure infused with TIP3P water combined with aqueous buffer spanning up till 20 Å encompassing sodium (Na^{+}) as well as chloride (Cl^{-}) ions both attaining concentrations measuring around 0The next step involved the preparation of systems following which they underwent an energetically minimized state and then equilibrated for a period of 5nano-seconds, so for starting the simulation process of protein-ligand complexes of **8c** and **8e** we used their top-scored conformations. The constraints and topologies of both derivatives were calculated by VMD plugin ForceField Toolkit (ffTK). The generated files were loaded to VMD to readily recite the protein–ligand complexes without errors and then conduct the simulation steps.

Supplementary S2. Binding free energy calculation

Binding free energy was calculated for docked complexes using MM-PBSA within MMPBSA.py module integrated with AMBER18 software package. An estimate of the system's net energy was obtained by using this equation and analysing data from processing 100 frames of the total trajectories.

$$\Delta G_{Binding} = \Delta G_{Complex} - \Delta G_{Receptor} - \Delta G_{Inhibitor}$$

The previous terms required multiple calculations involving Van der Waals forces and electrostatics; this term also needs an accurate calculation for internal energies derived through mechanism with a polar contribution towards solvation.

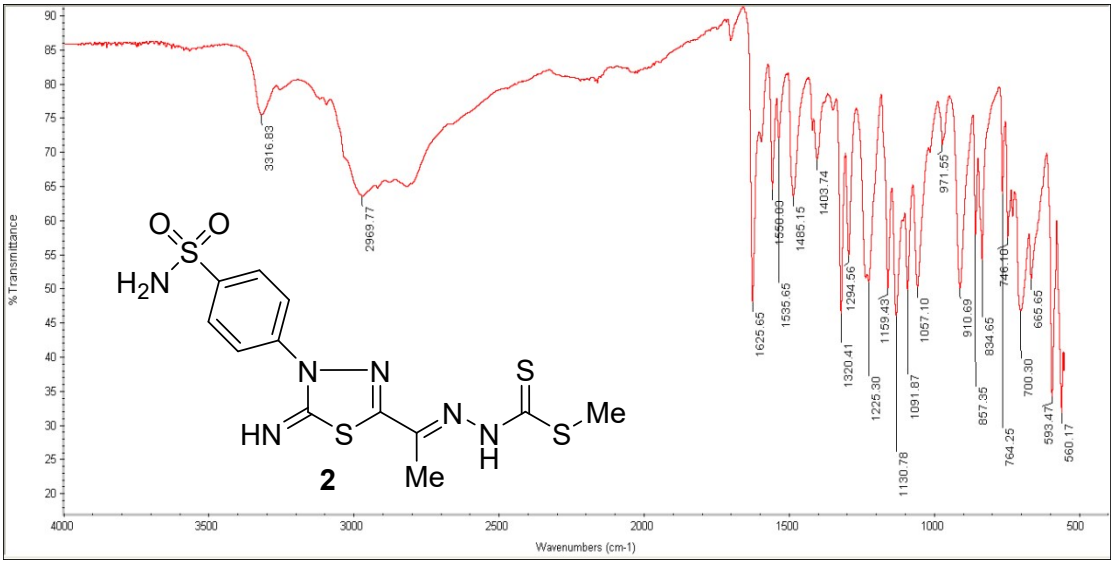

Figure S1. IR spectrum of compound **2**.

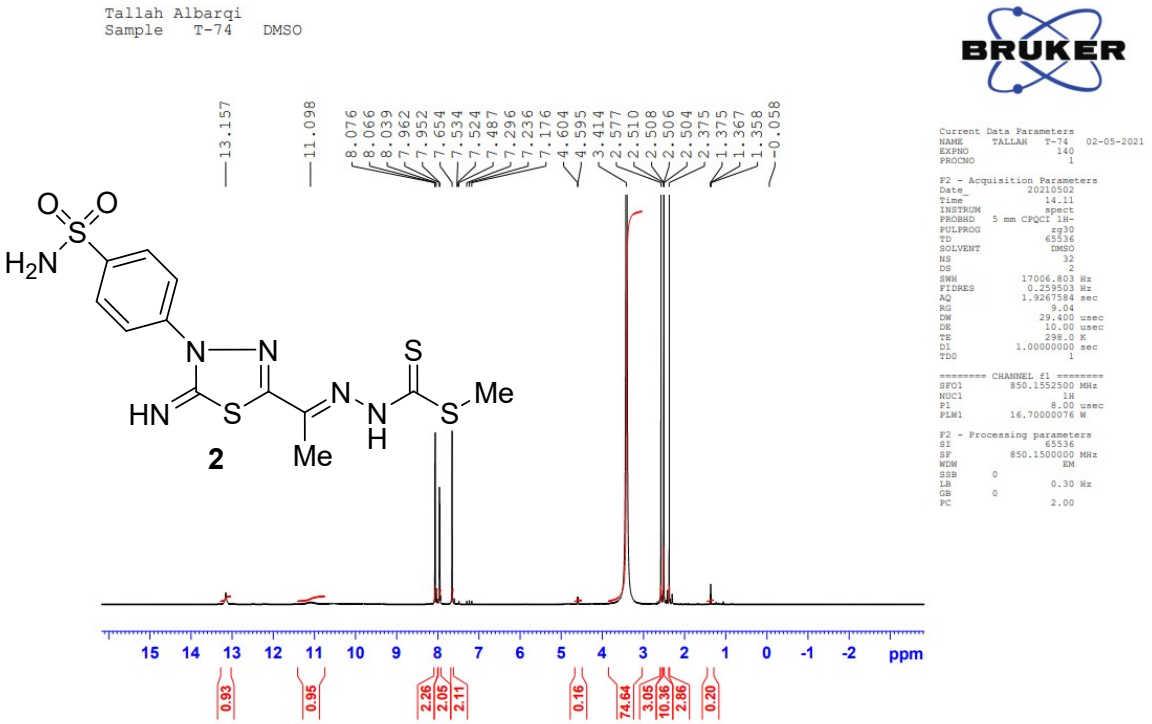

Figure S2. ^{1}H-NMR spectrum of compound **2.**

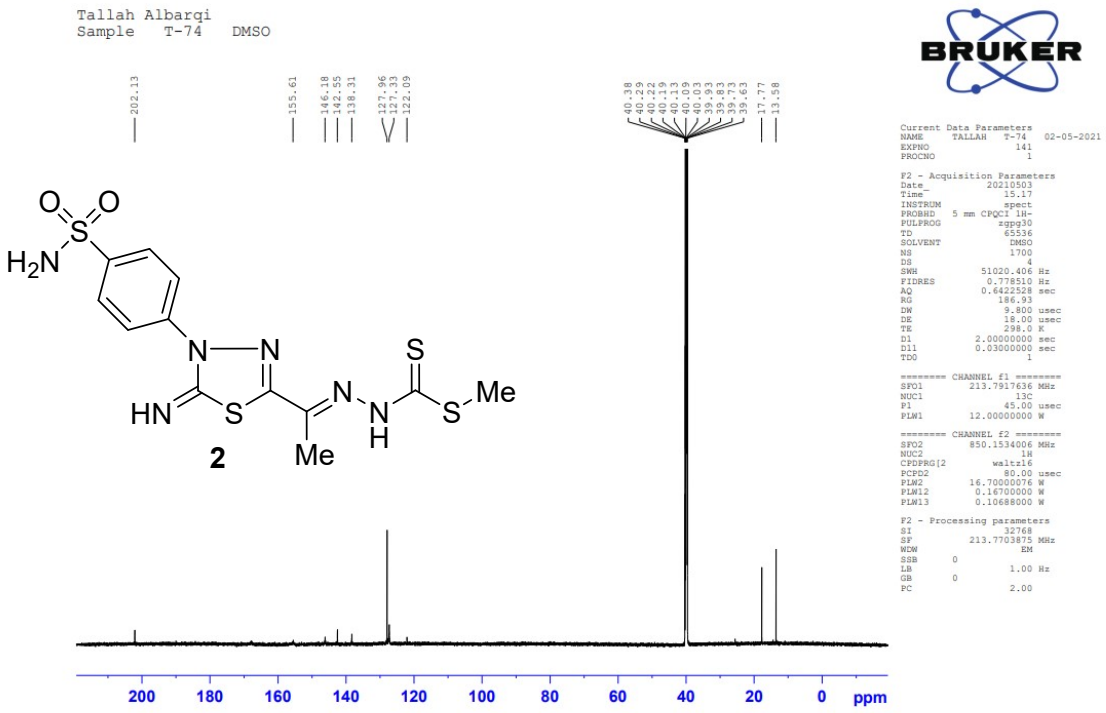

Figure S3. ^{13}C-NMR spectrum of compound **2**.

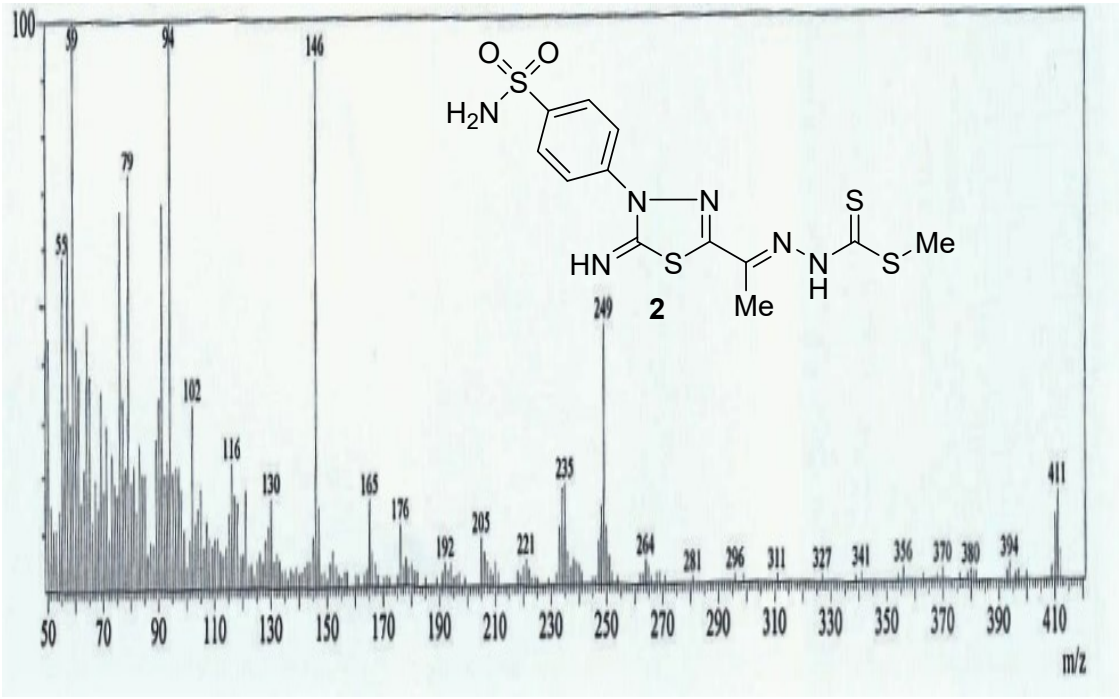

Figure S4. Mass spectrum of compound **2.**

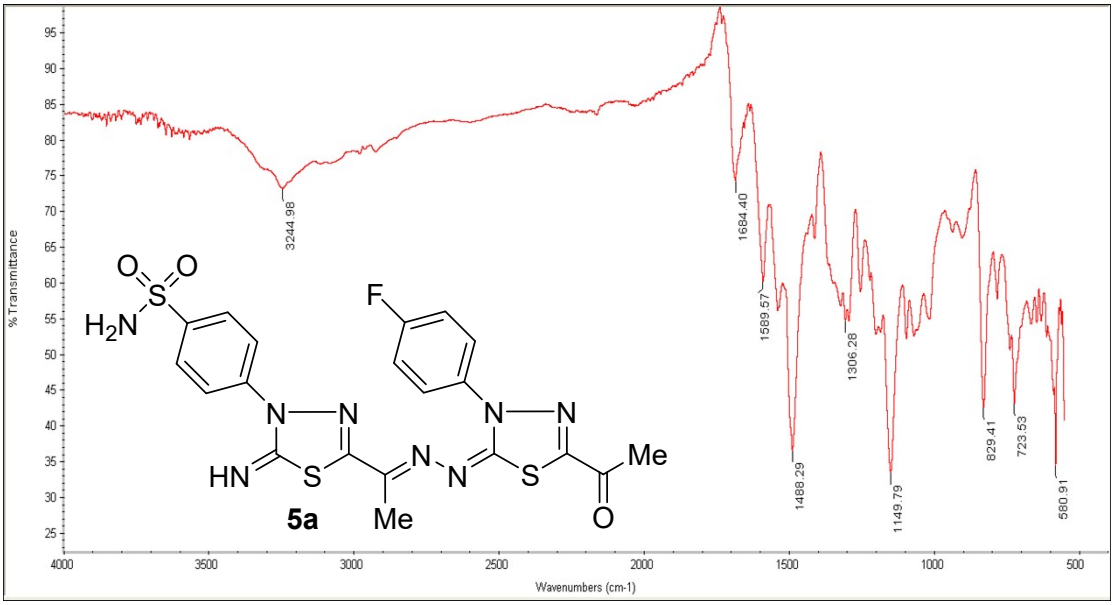

Figure S5. IR spectrum of compound **5a**.

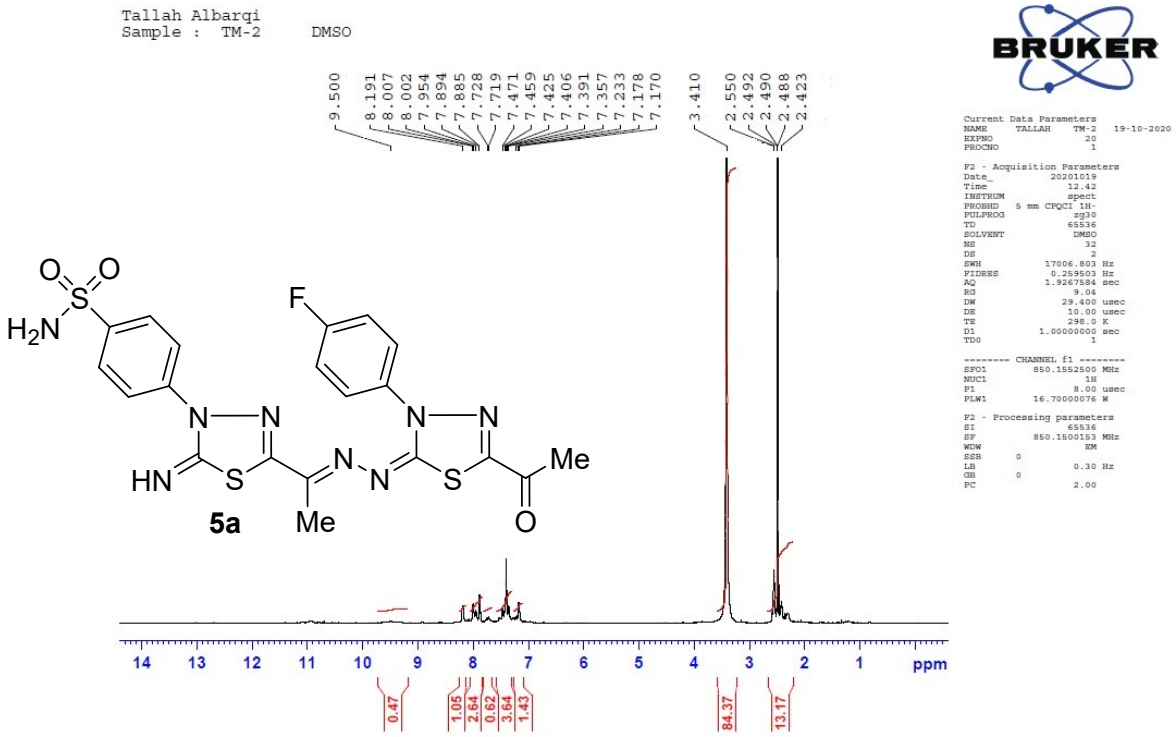

Figure S6. ^{1}H-NMR spectrum of compound **5a**.

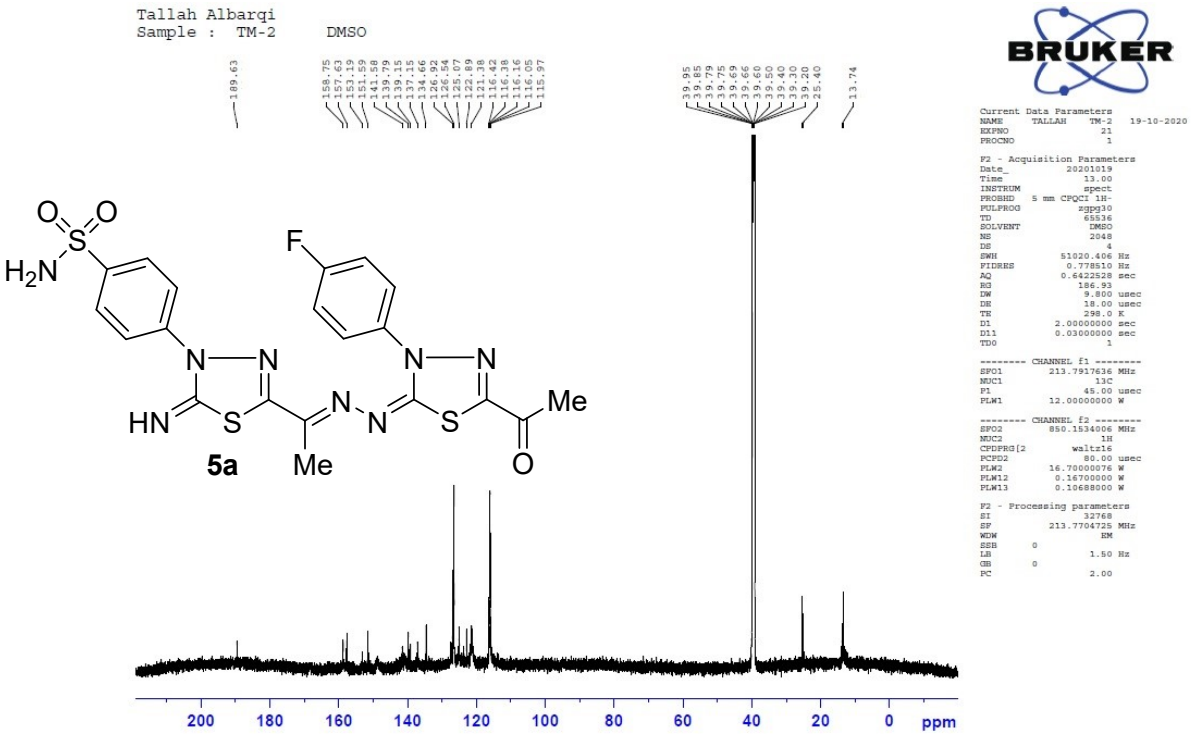

Figure S7. ^{13}C-NMR spectrum of compound **5a**.

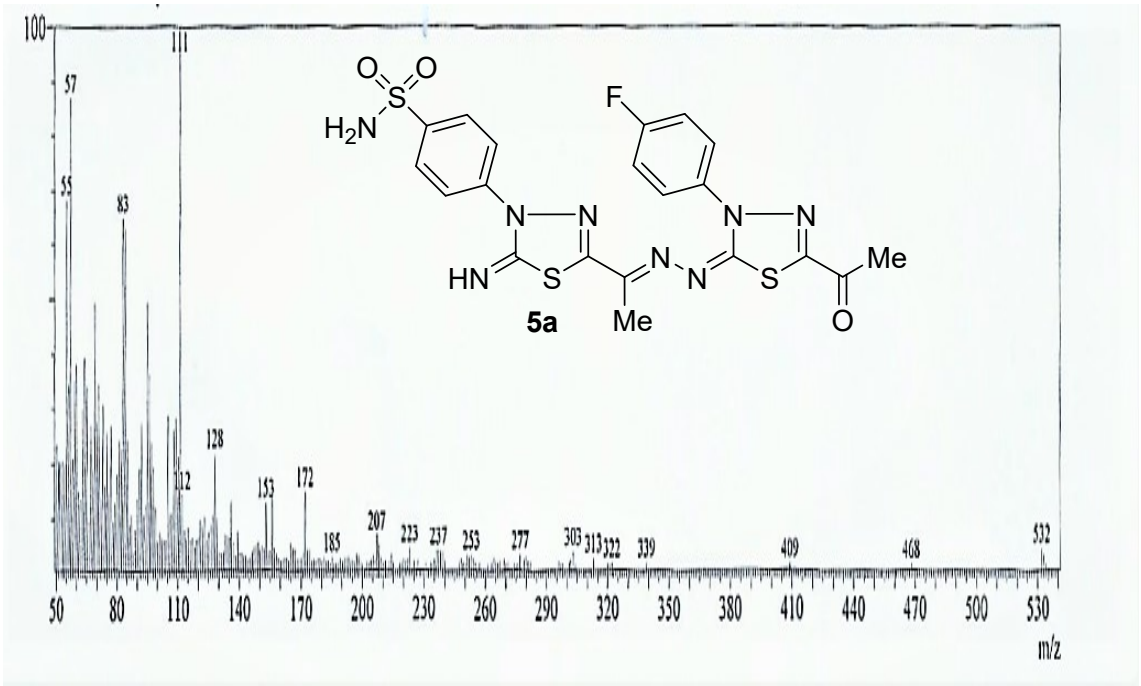

Figure S8. Mass spectrum of compound **5a.**

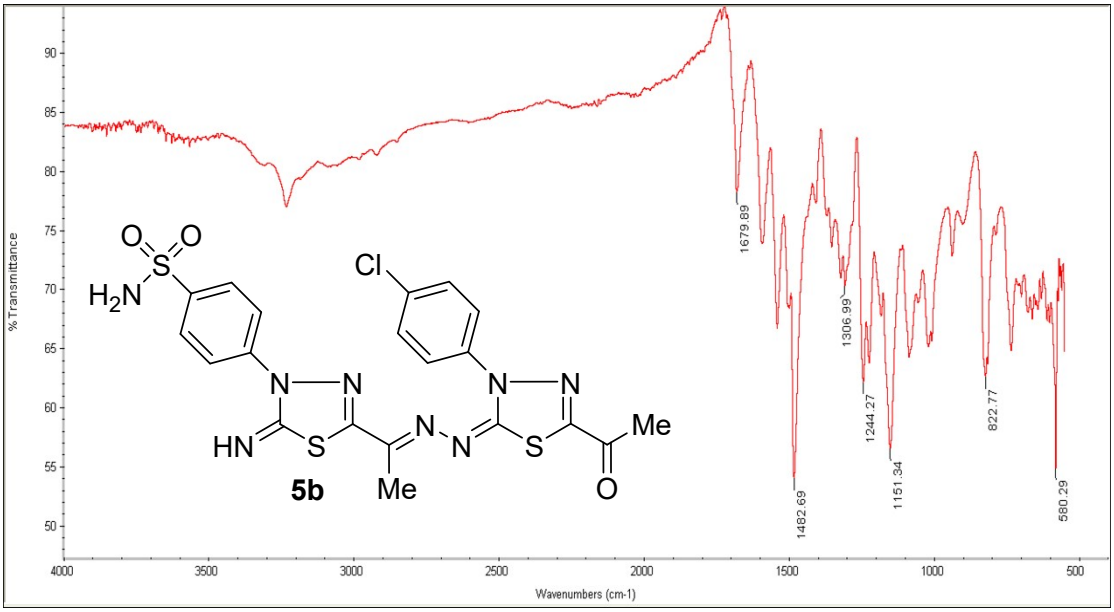

Figure S9. IR spectrum of compound **5b**.

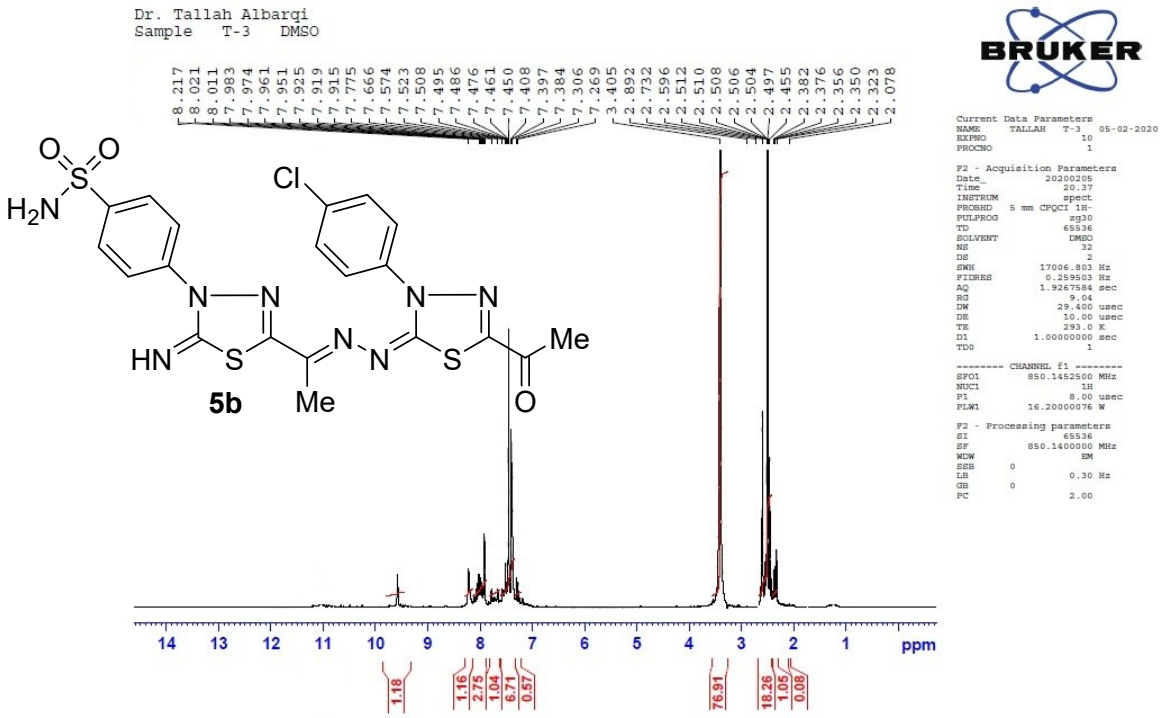

Figure S10. ^{1}H-NMR spectrum of compound **5b**.

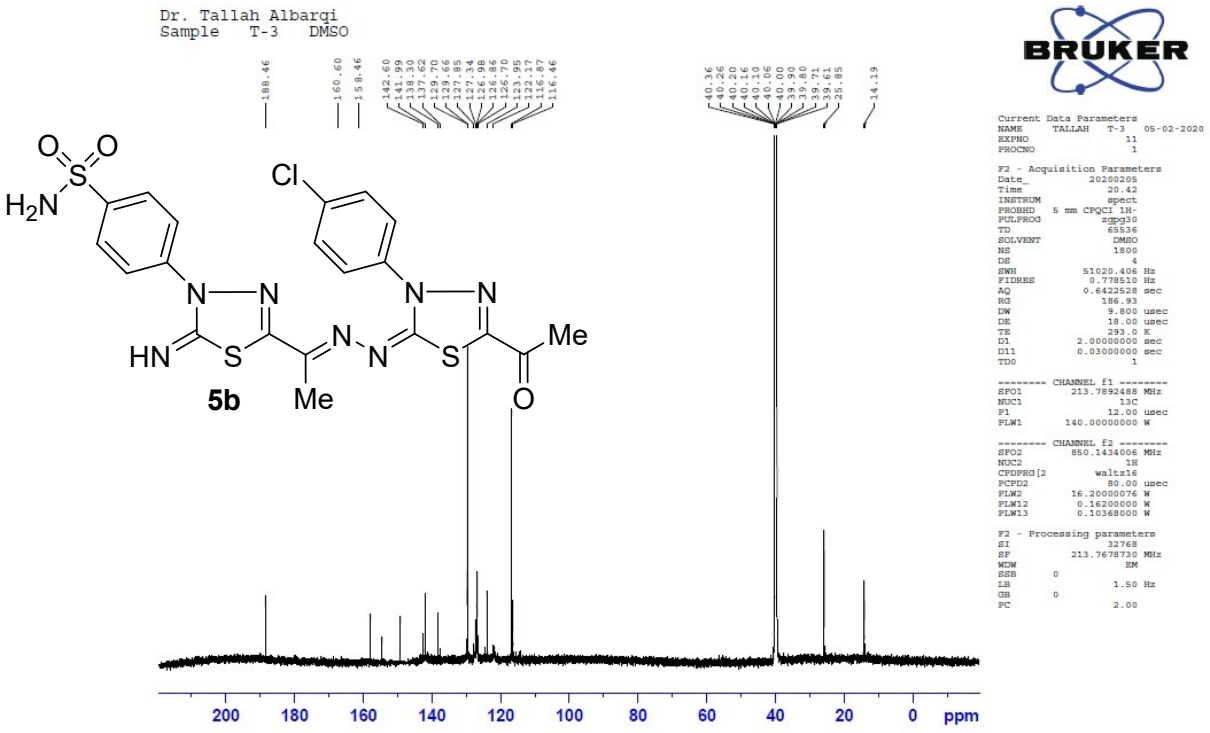

Figure S11. ^{13}C-NMR spectrum of compound **5b**.

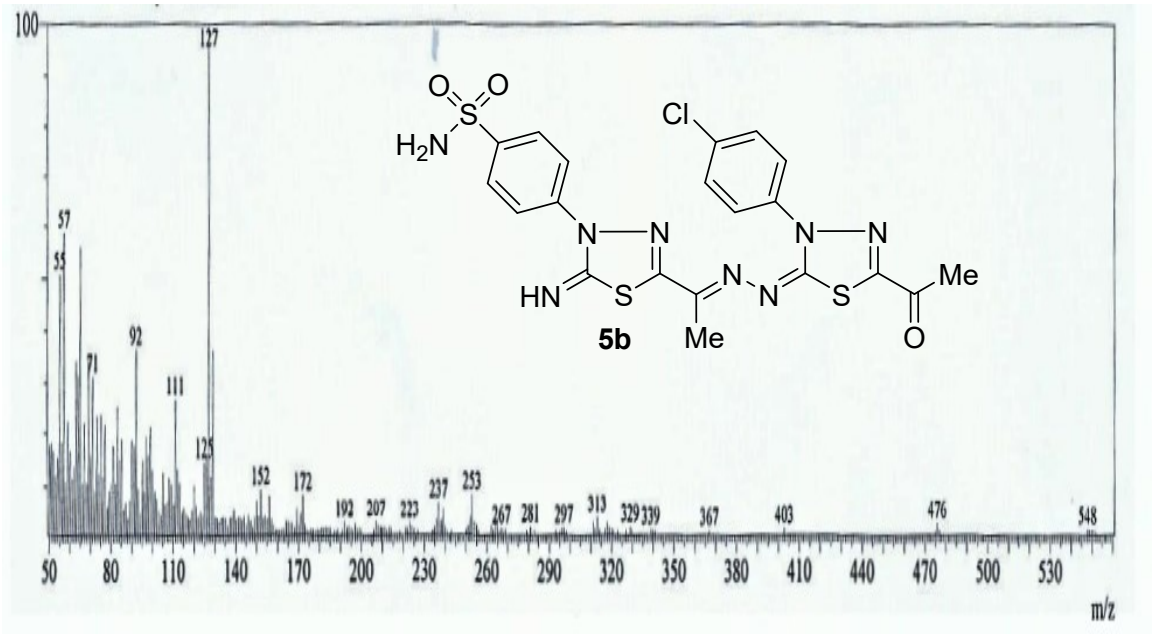

Figure S12. Mass spectrum of compound **5b**.

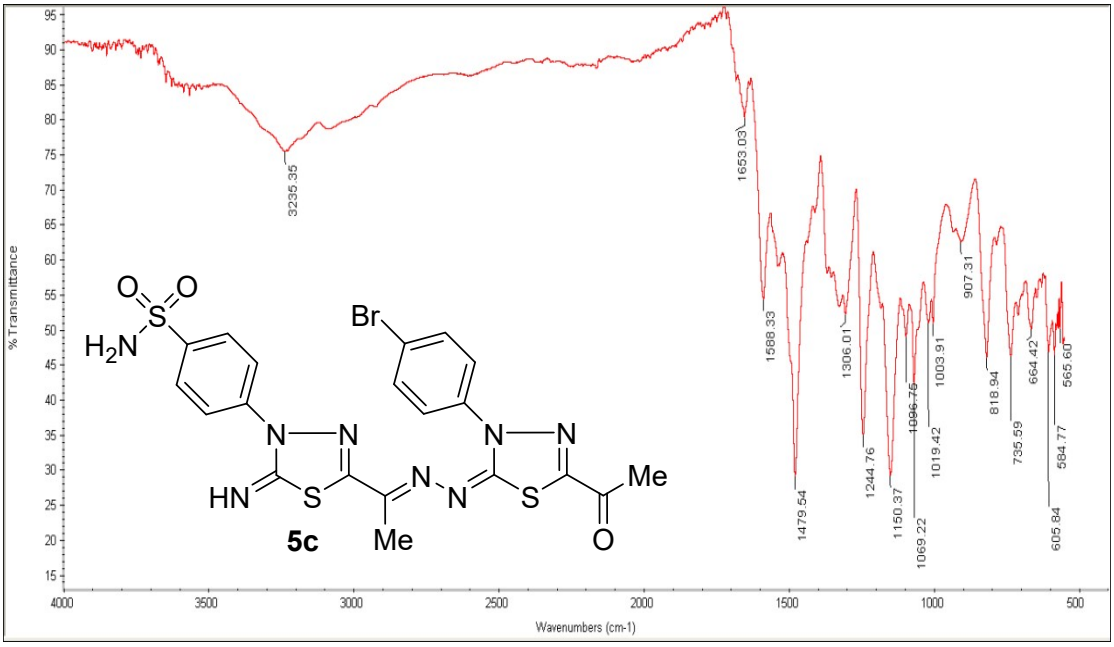

Figure S13. IR sepectrum of compound **5c**.

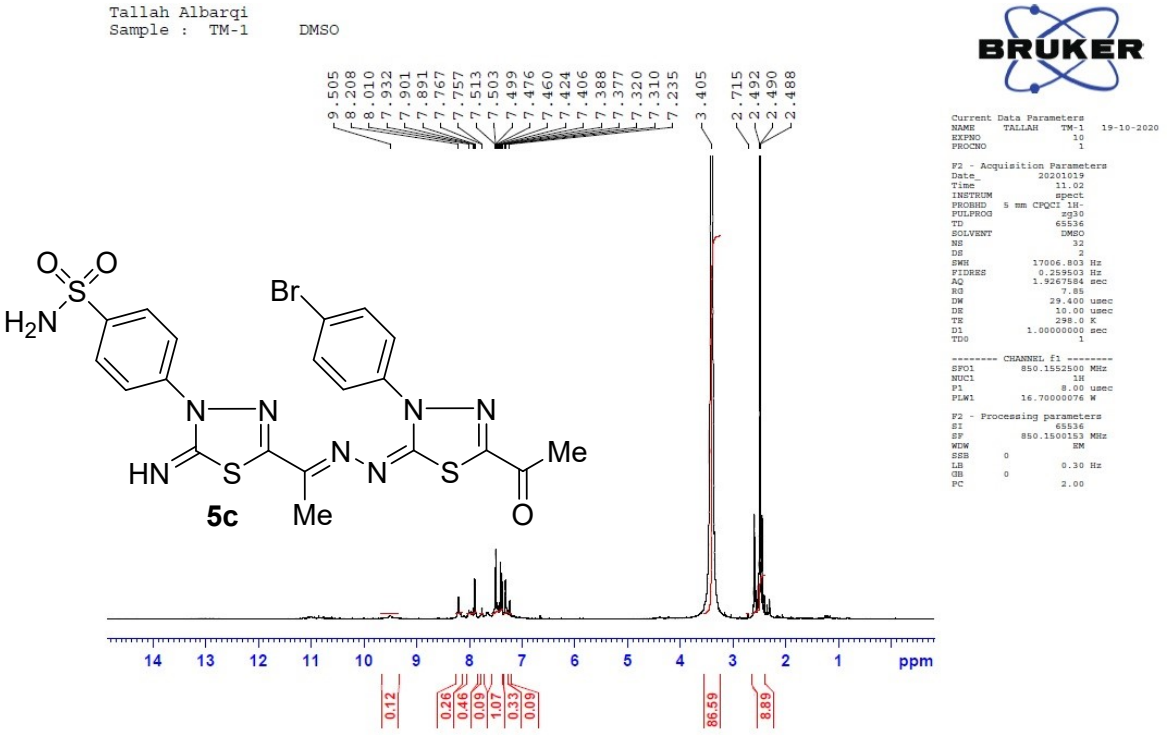

Figure S14. ^{1}H-NMR spectrum of compound **5c**.

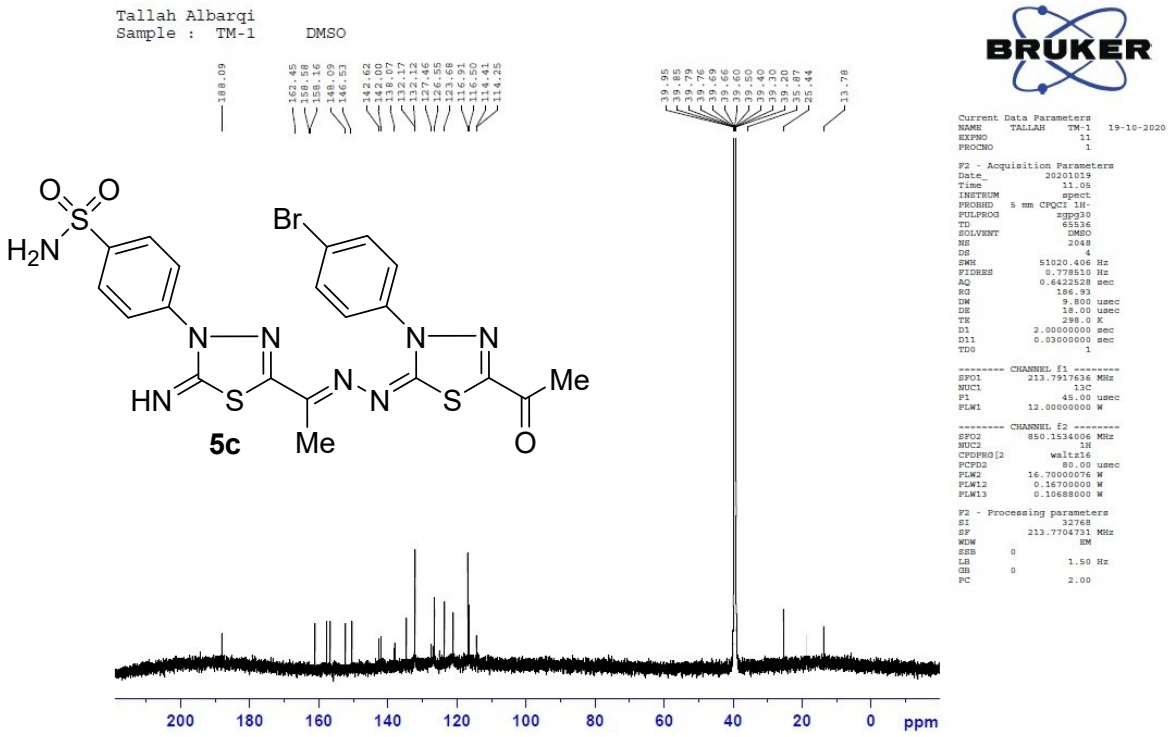

Figure S15. ^{13}C-NMR spectrum of compound **5c**.

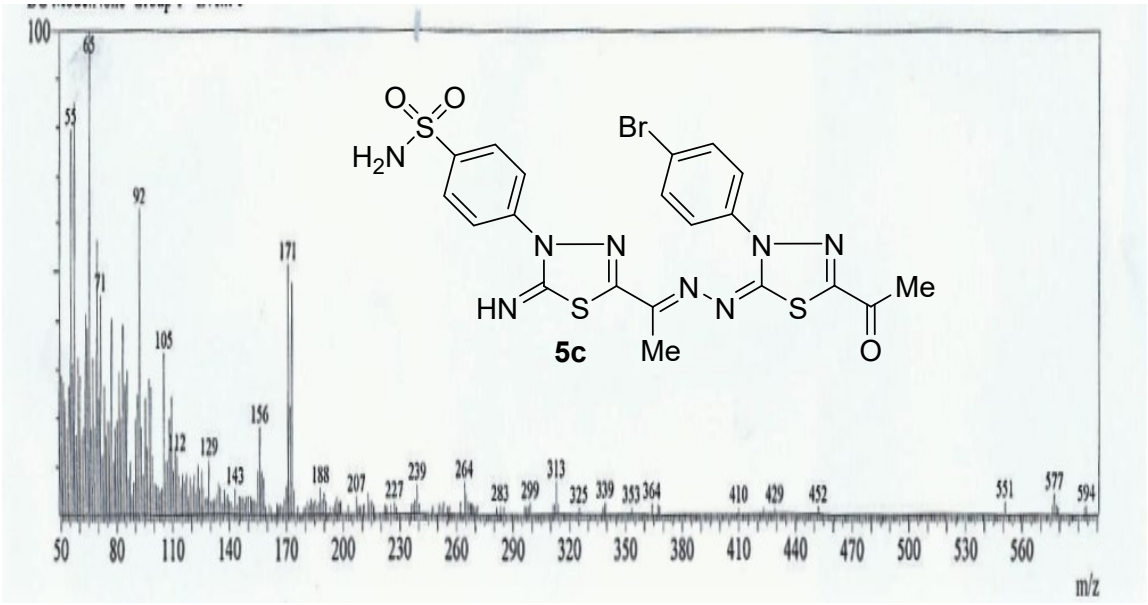

Figure S16. Mass spectrum of compound **5c**

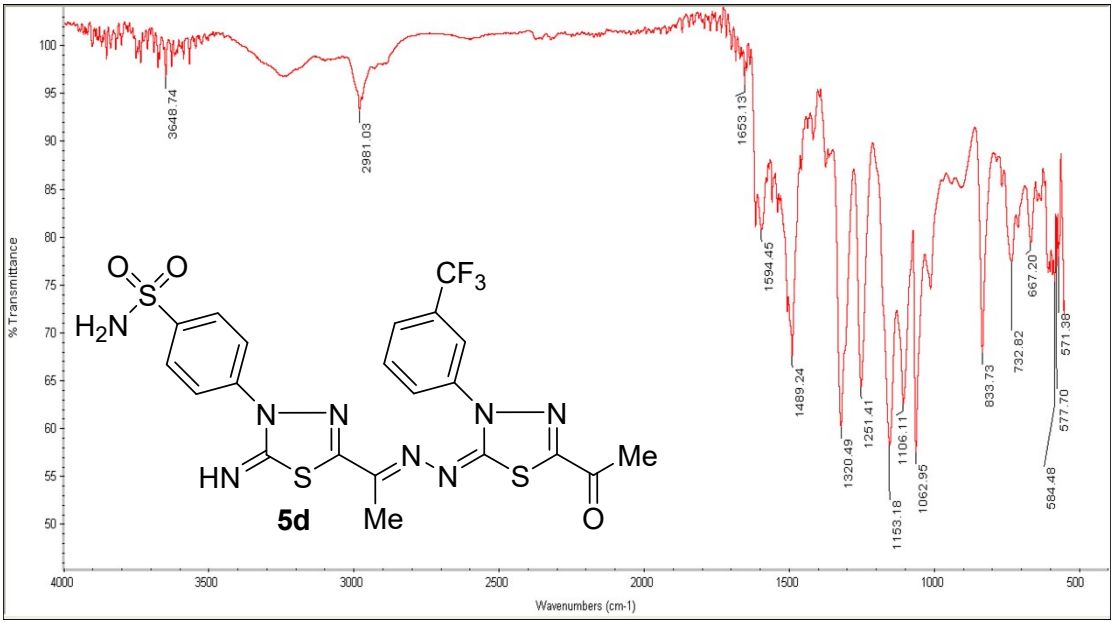

Figure S17. IR spectrum of compound **5d**.

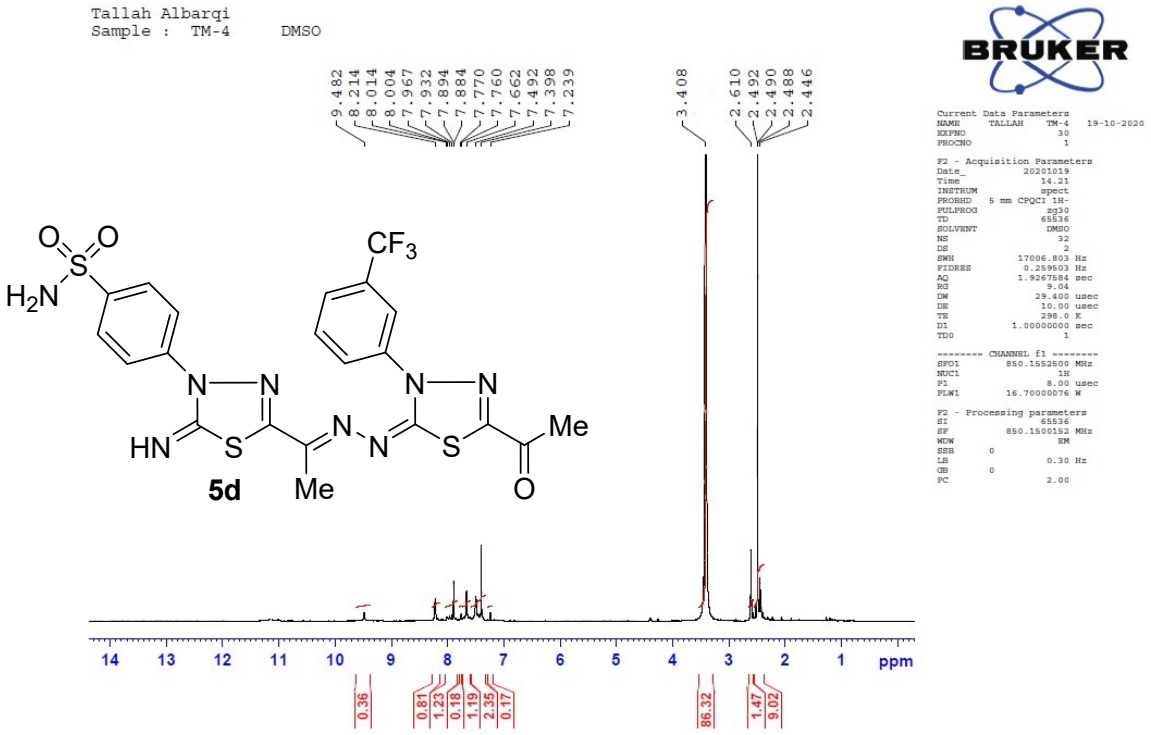

Figure S18. ^{1}H-NMR spectrum of compound **5d**.

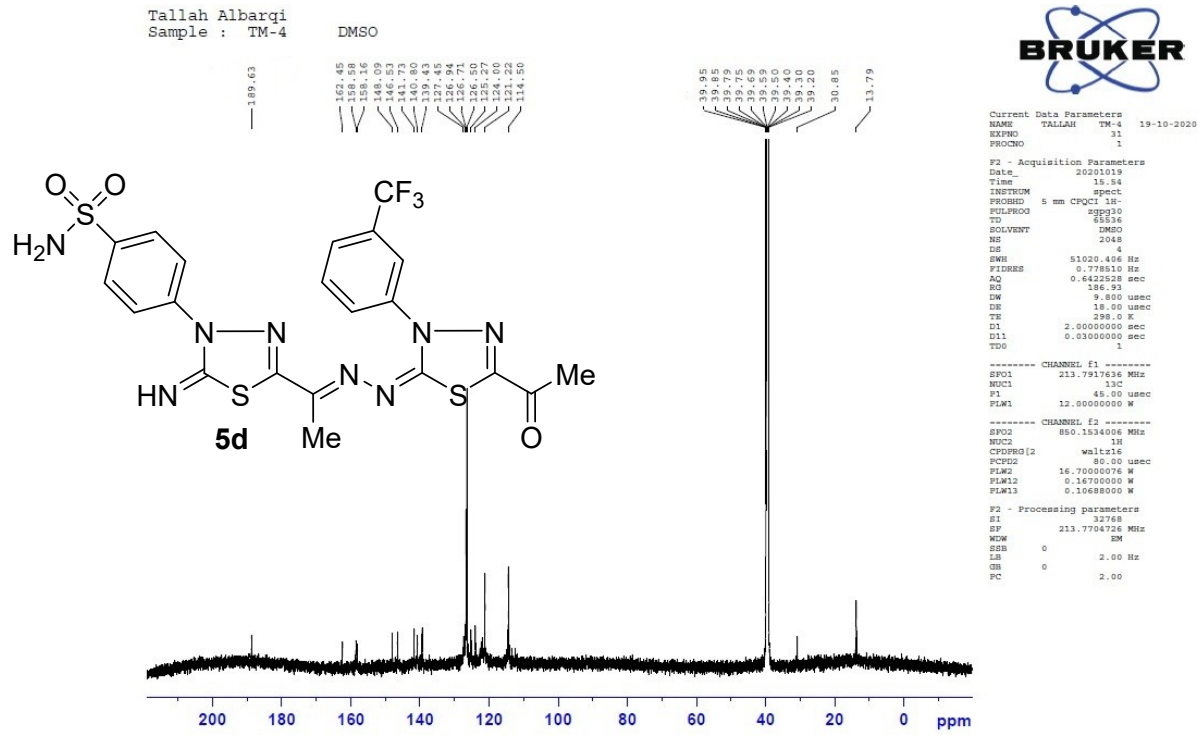

Figure S19. ^{13}C-NMR spectrum of compound **5d**.

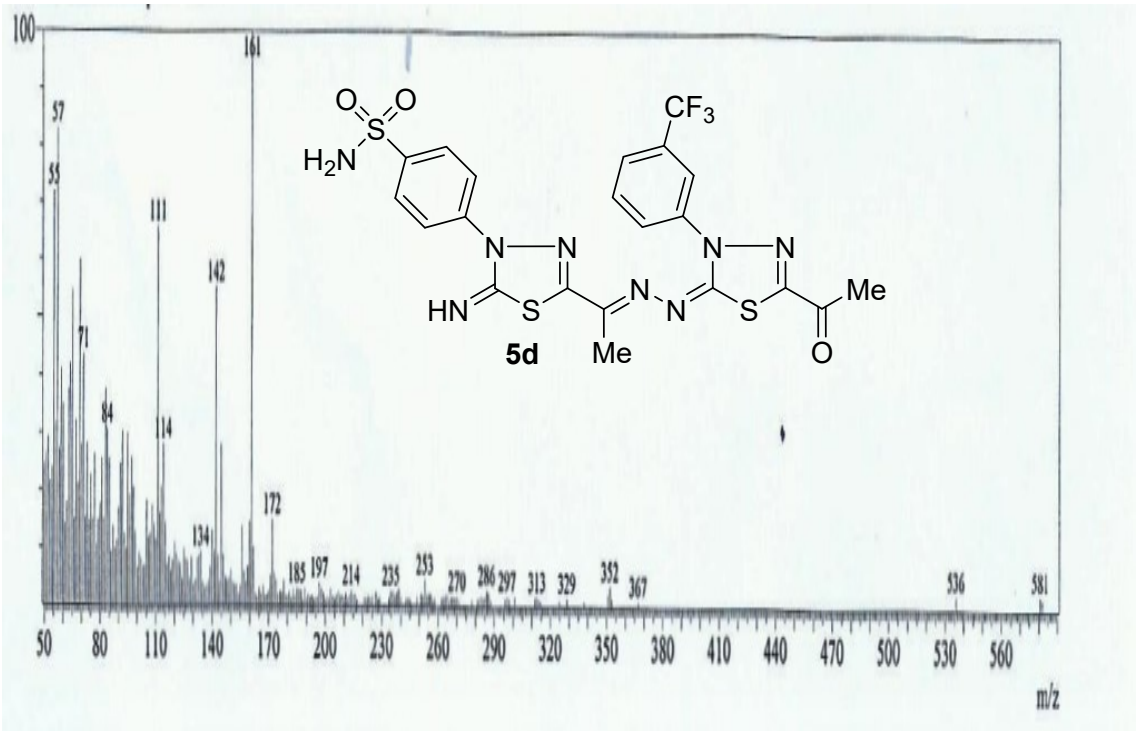

Figure S20. Mass spectrum of compound **5d**

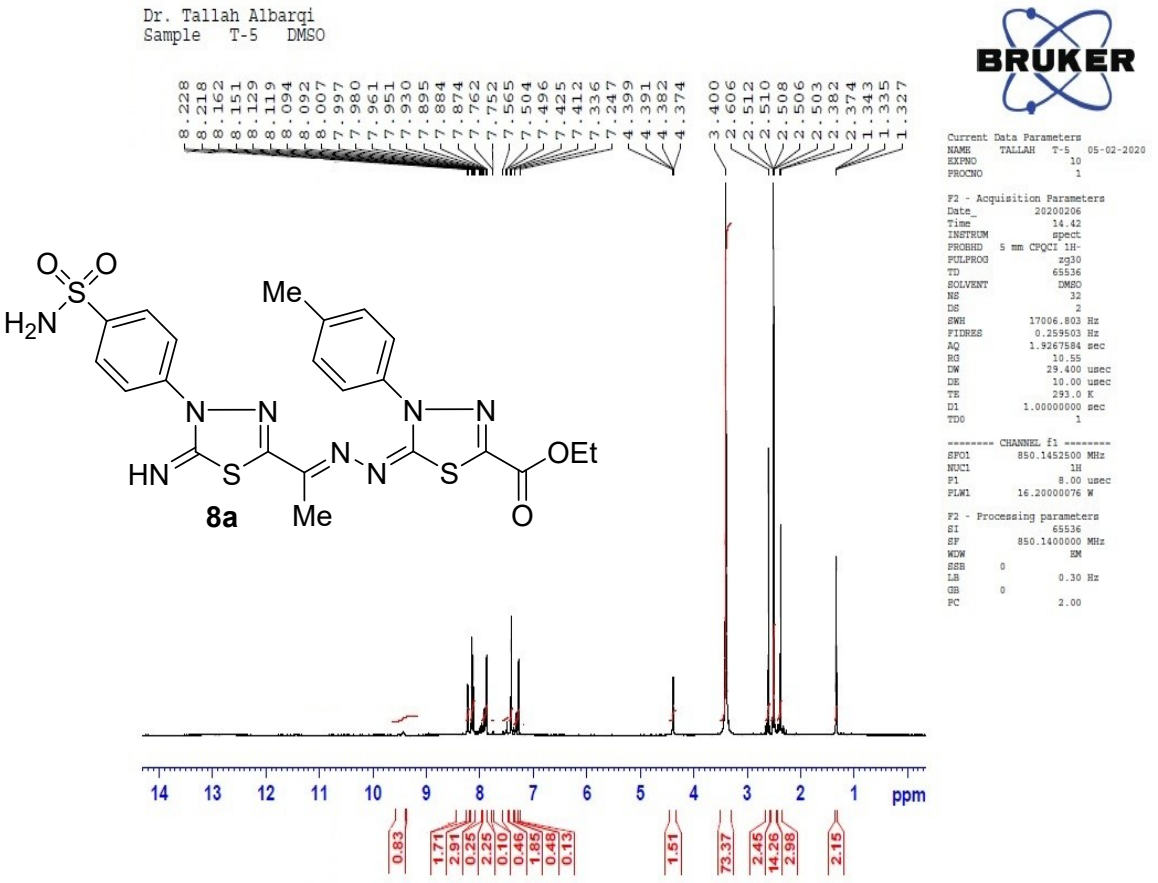

Figure S21. ^{1}H-NMR spectrum of compound **8a**.

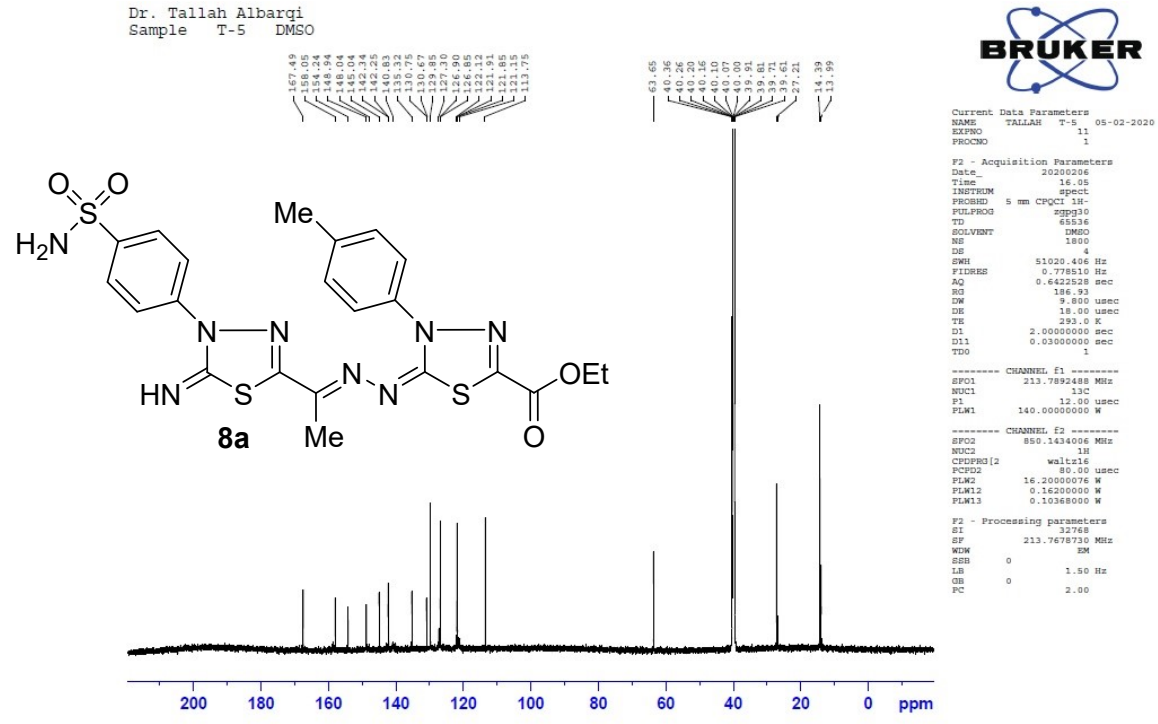

Figure S22. ^{13}C-NMR spectrum of compound **8a**.

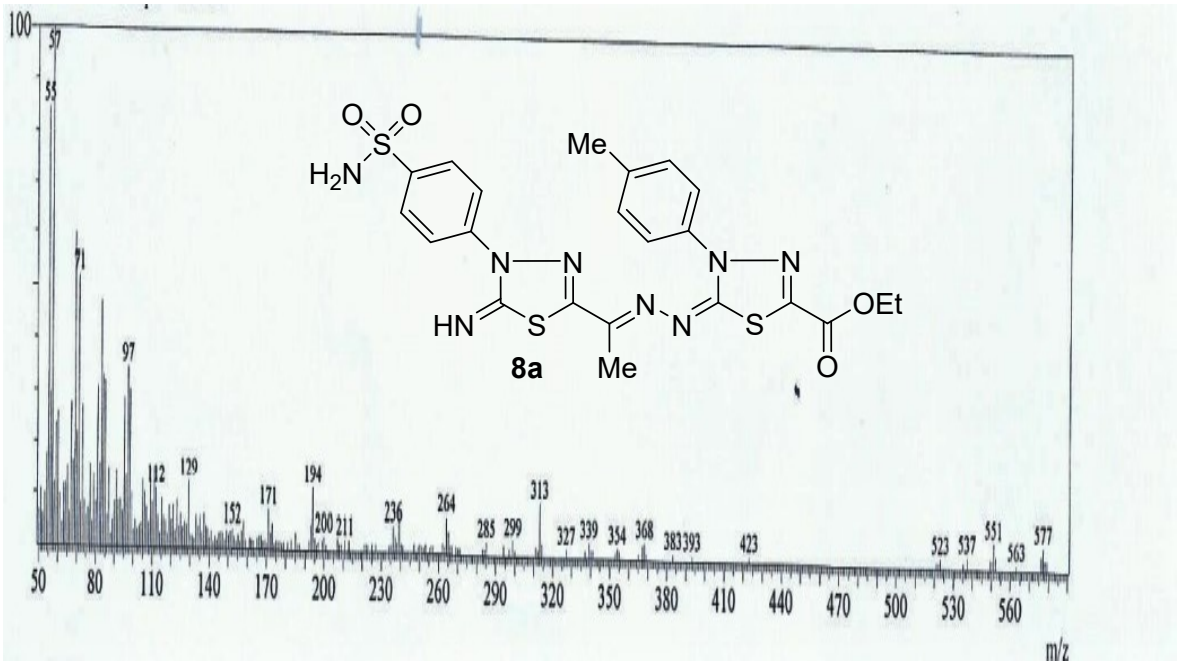

Figure S23. Mass spectrum of compound **8a.**

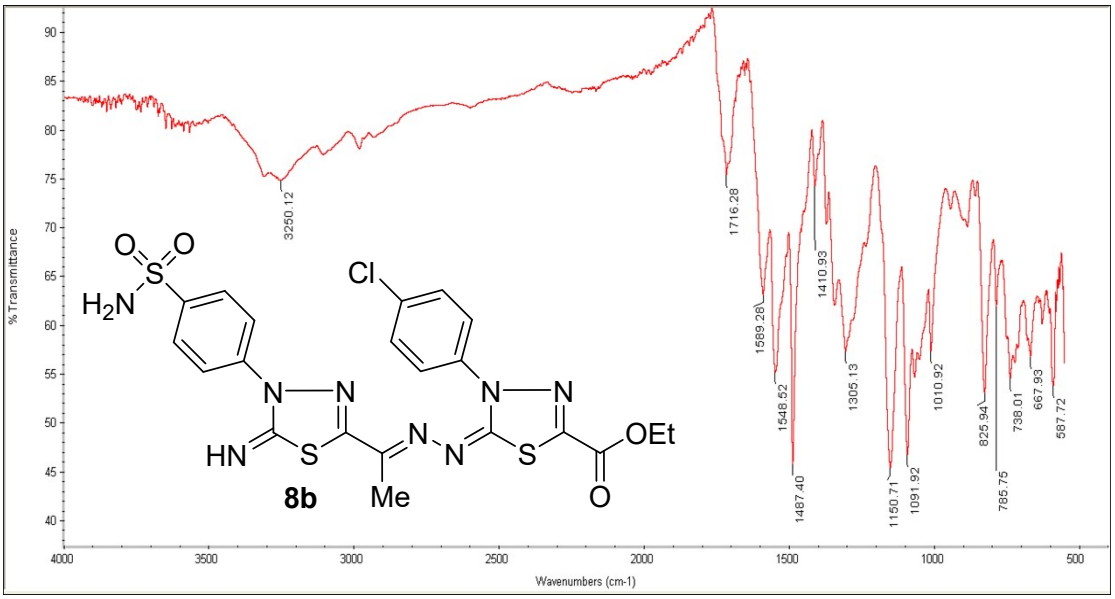

Figure S24. IR spectrum of compound **8b**.

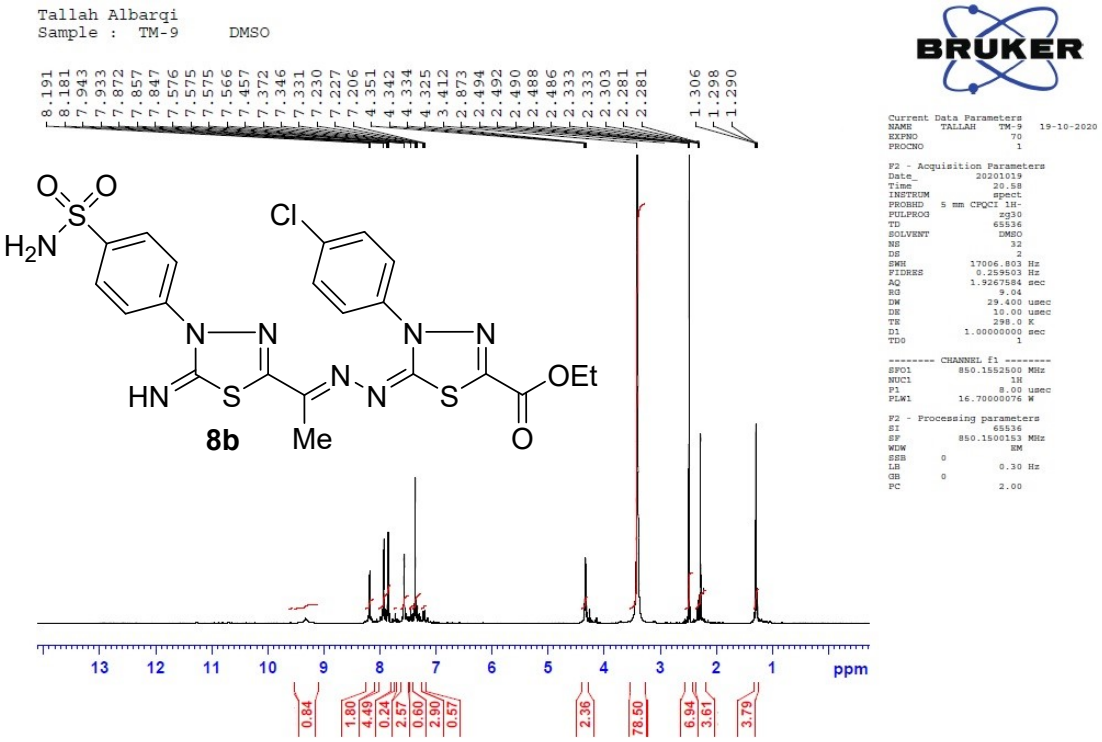

Figure S25. [1]H-NMR spectrum of compound **8b**.

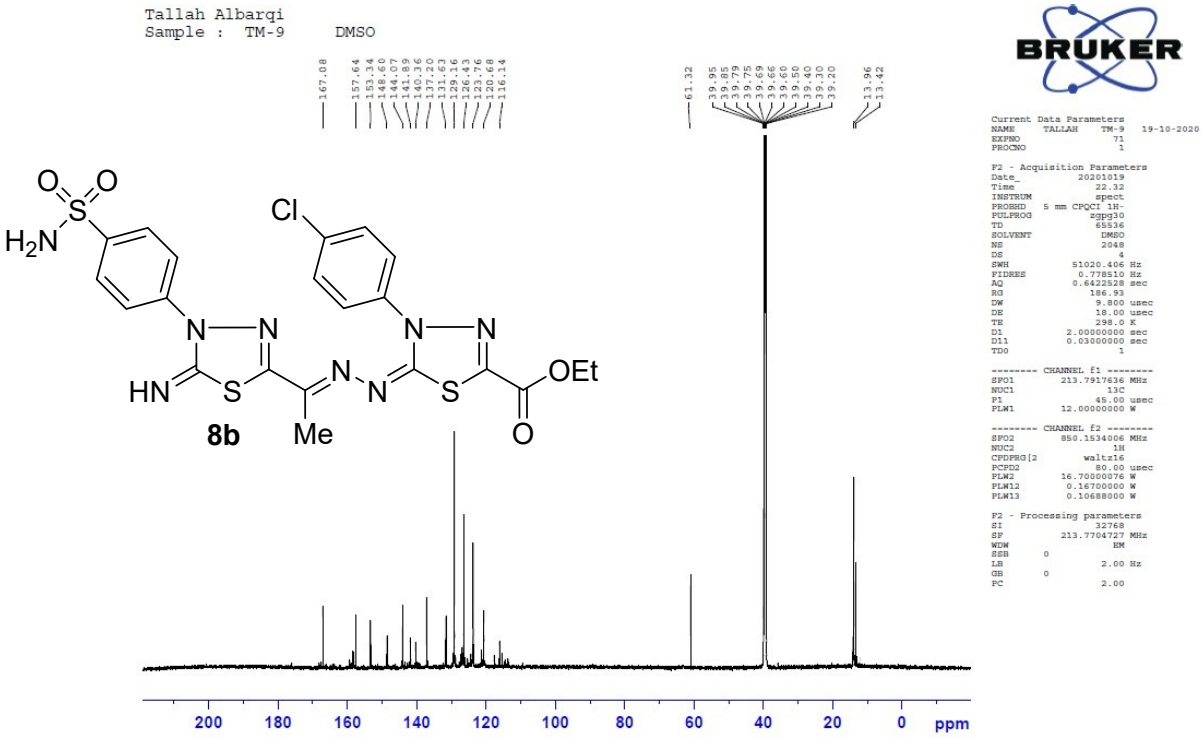

Figure S26. [13]C-NMR spectrum of compound **8b**.

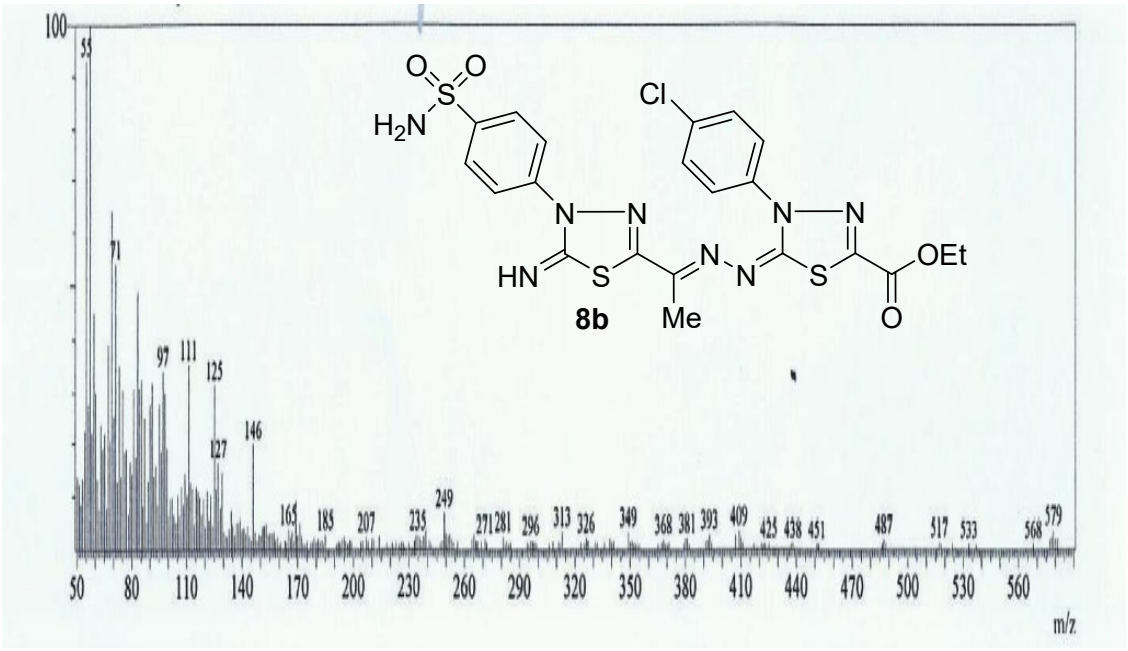

Figure S27. Mass spectrum of compound **8b.**

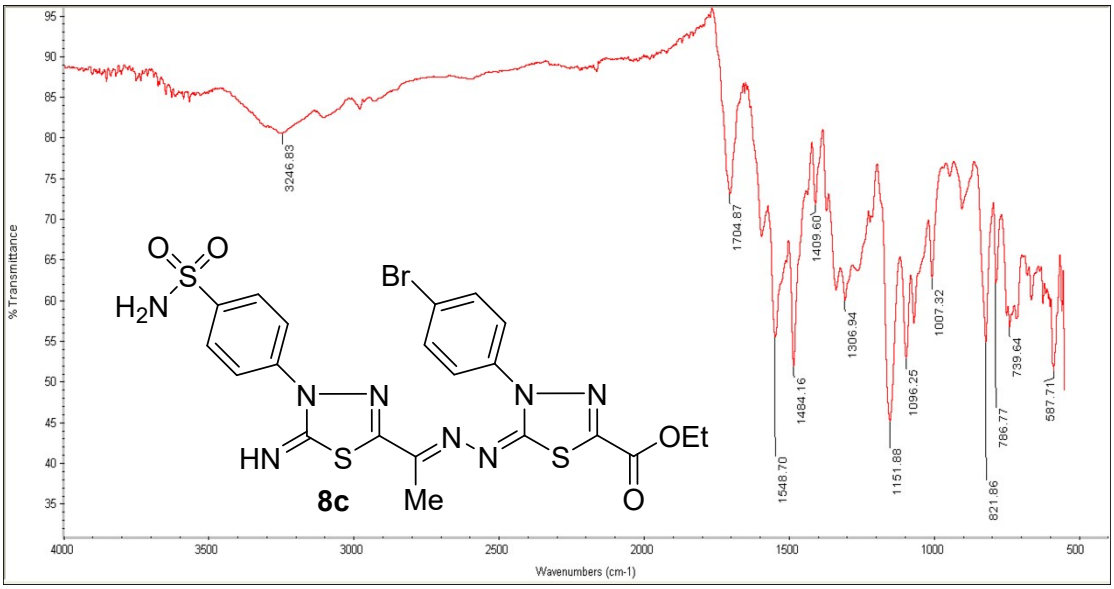

Figure S28. IR spectrum of compound **8c**.

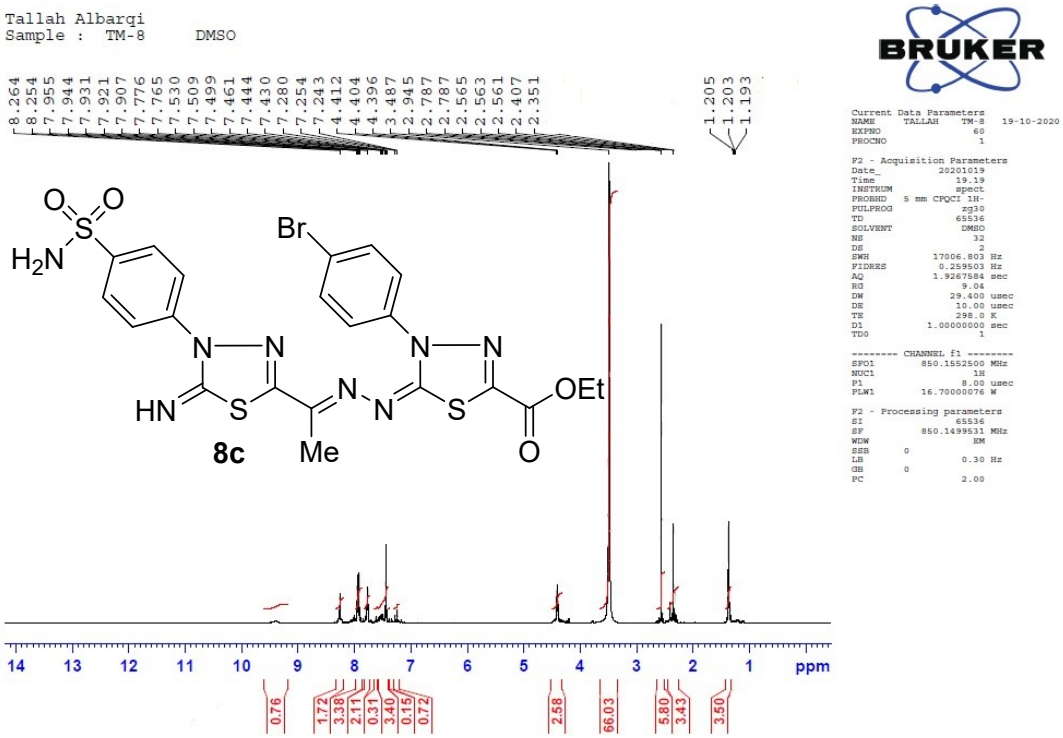

Figure S29. [1]H-NMR spectrum of compound **8c**.

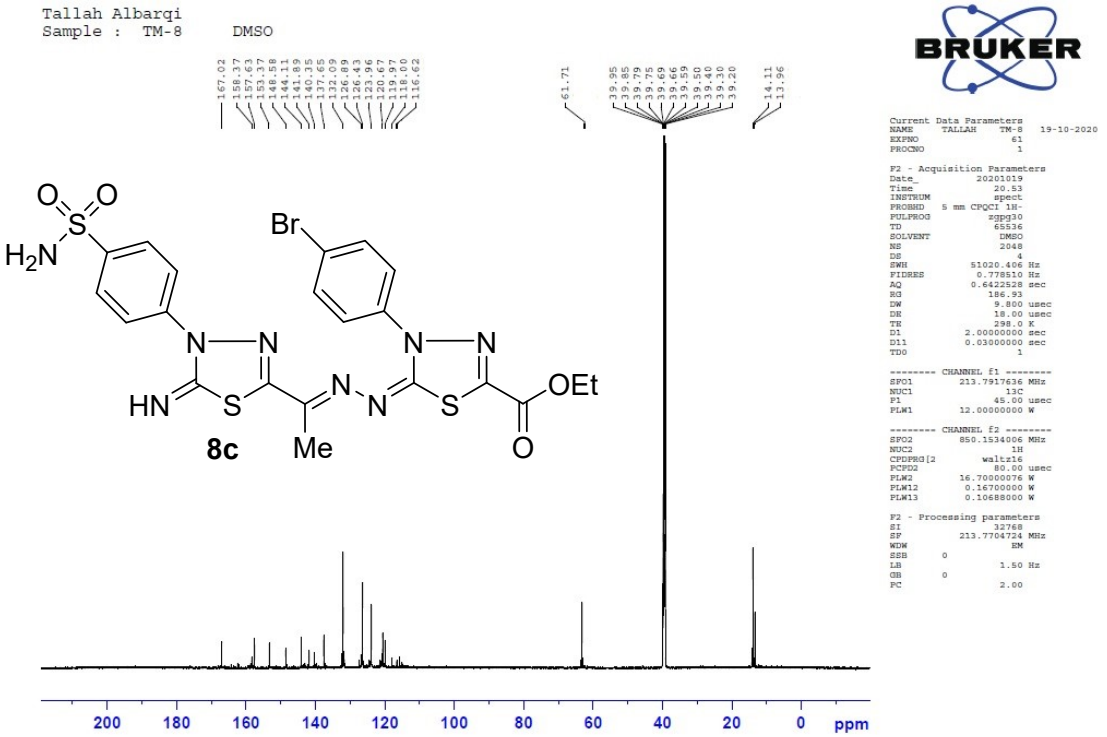

Figure S30. [13]C-NMR spectrum of compound **8c**.

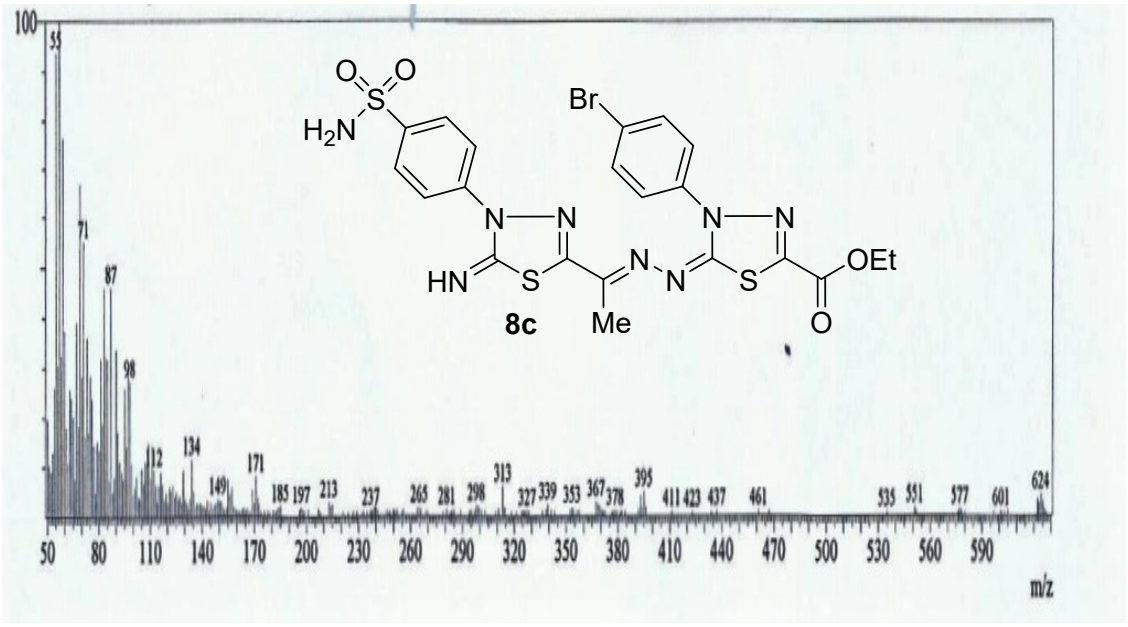

Figure S31. Mass spectrum of compound **8c.**

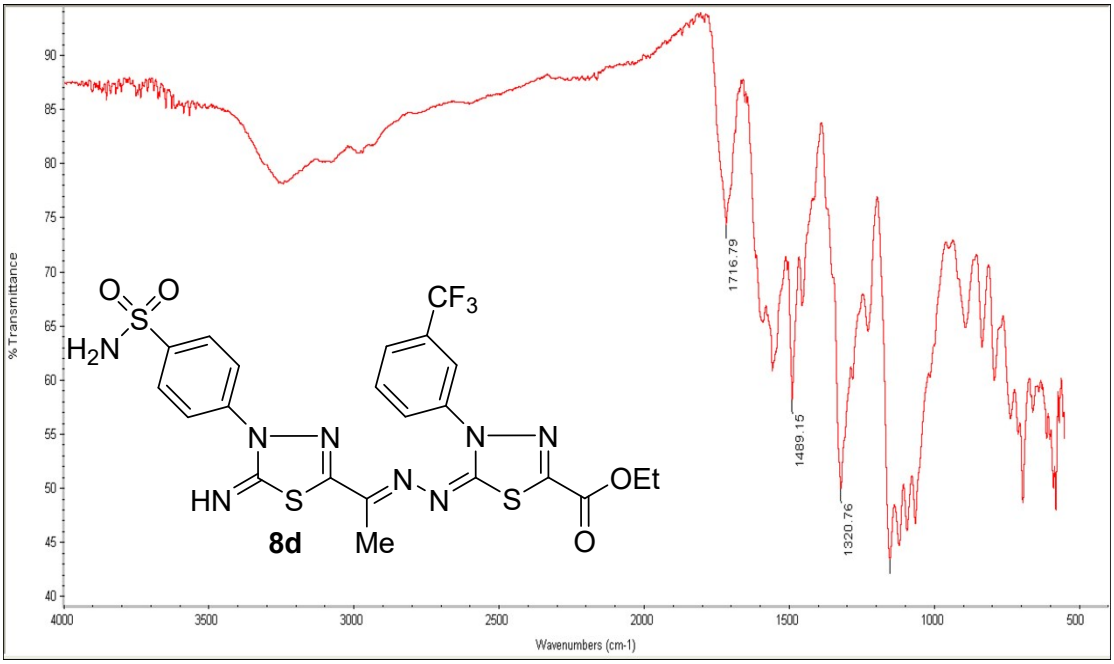

Figure S32. IR spectrum of compound **8d**.

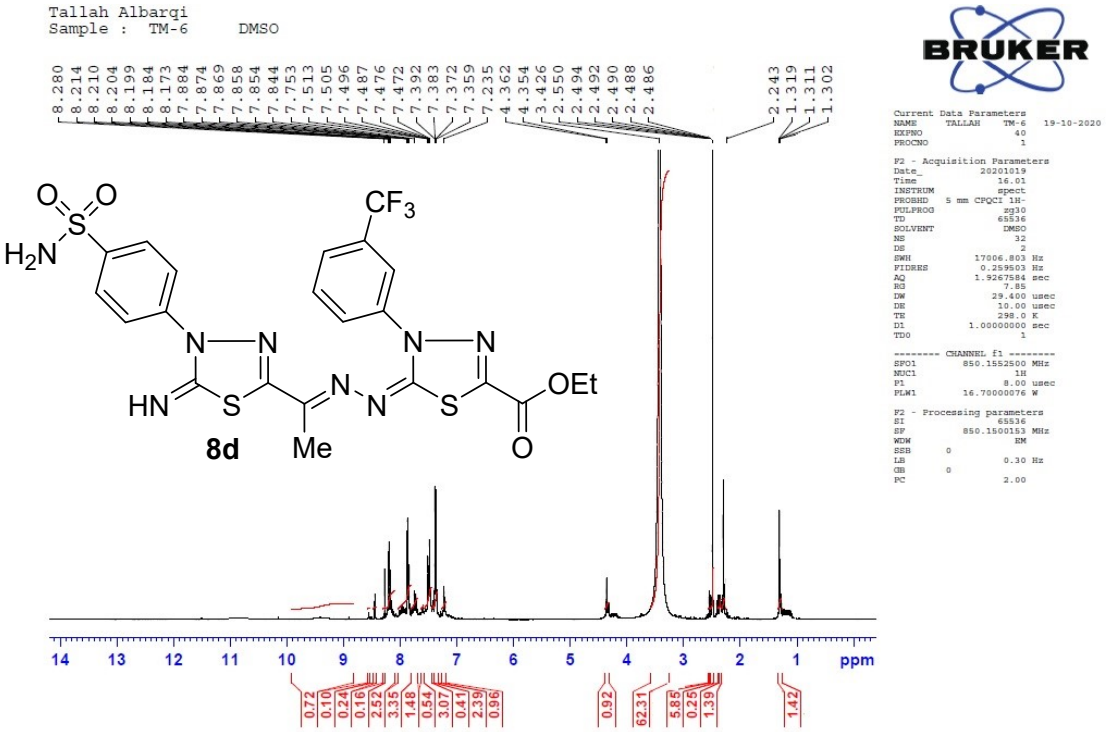

Figure S33. [1]H-NMR spectrum of compound **8d**.

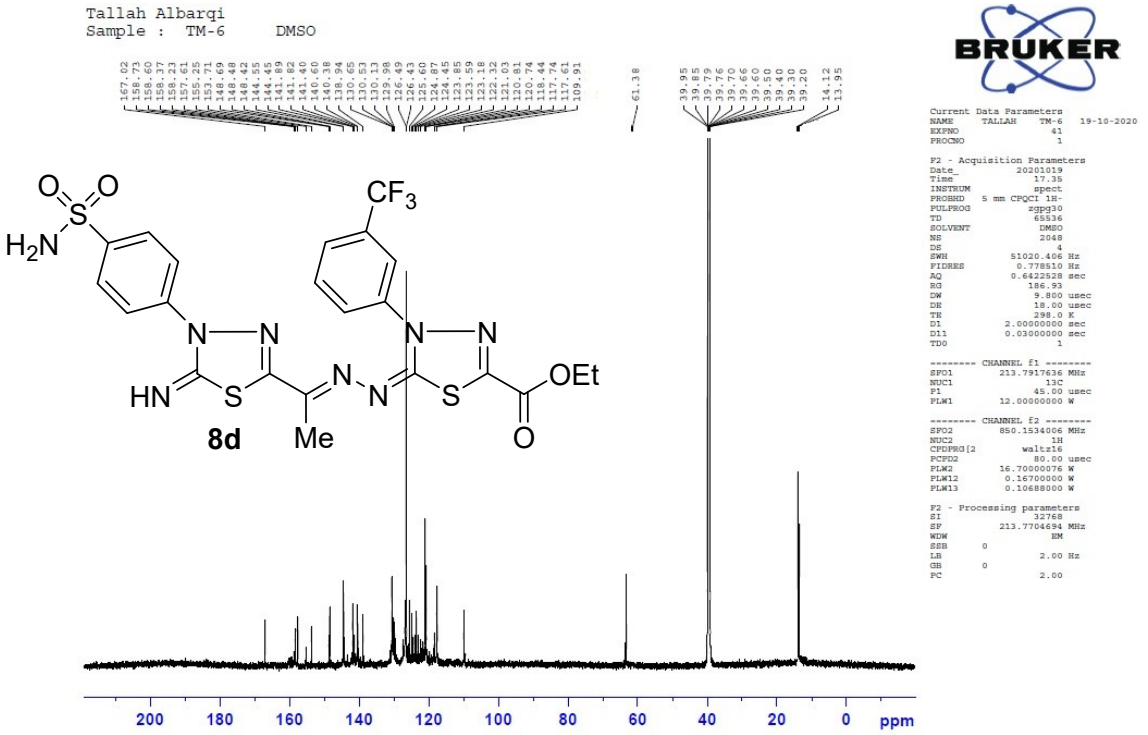

Figure S34. [13]C-NMR spectrum of compound **8d**.

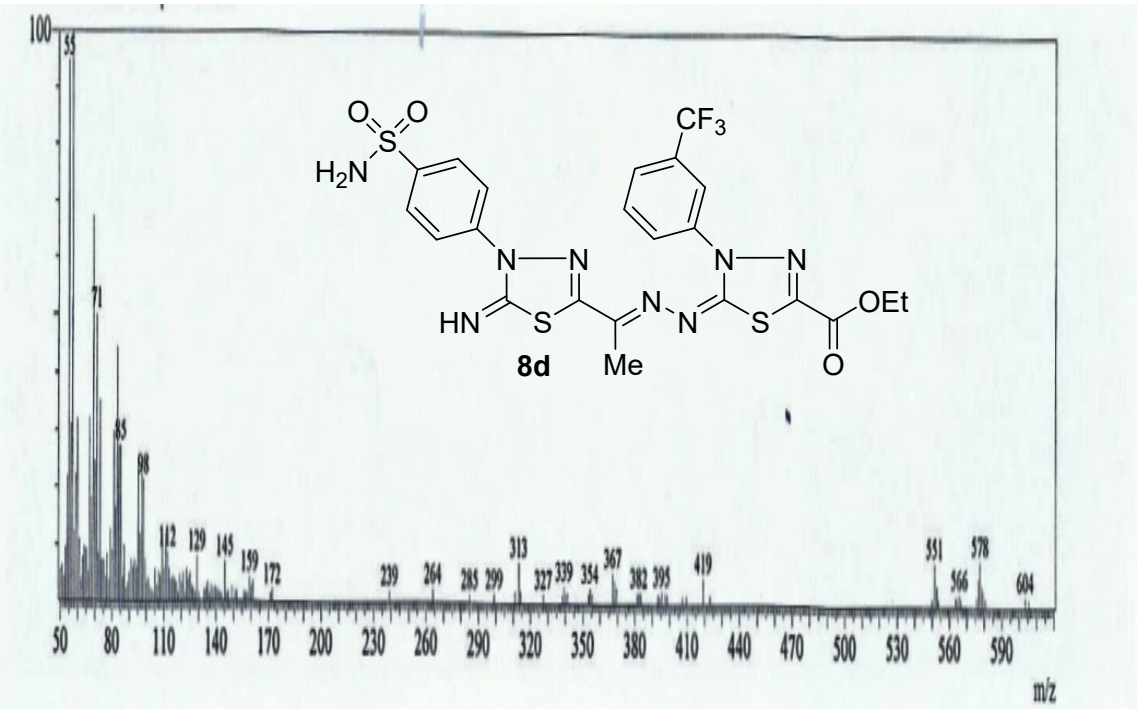

Figure S35. Mass spectrum of compound **8d.**

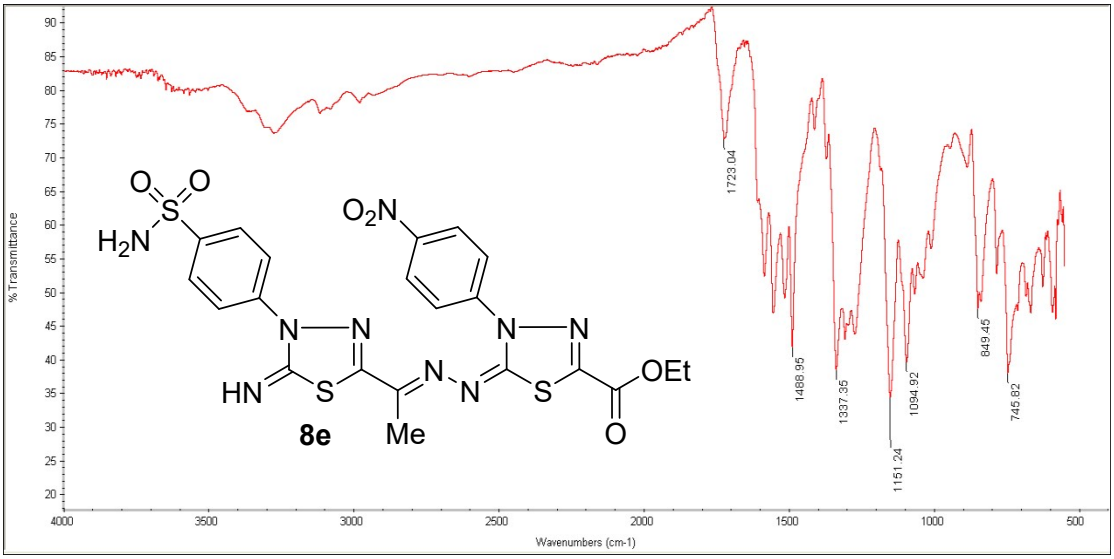

Figure S36. IR spectrum of compound **8e**.

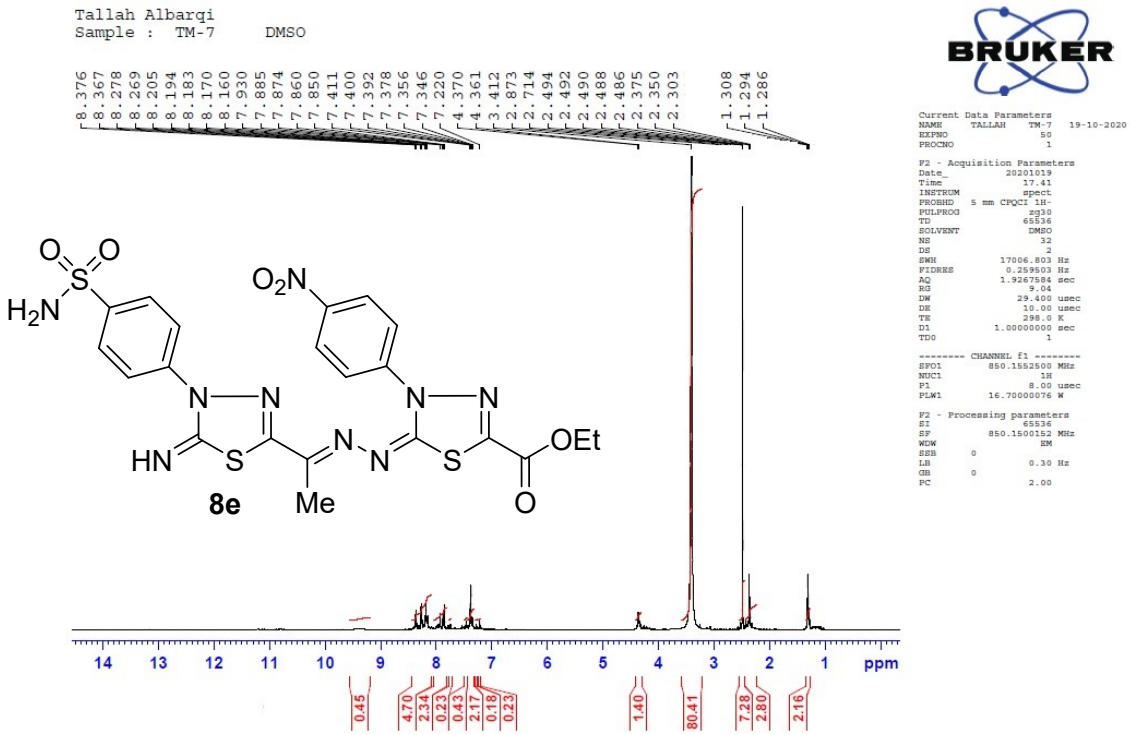

Figure S37. ^{1}H-NMR spectrum of compound **8e**.

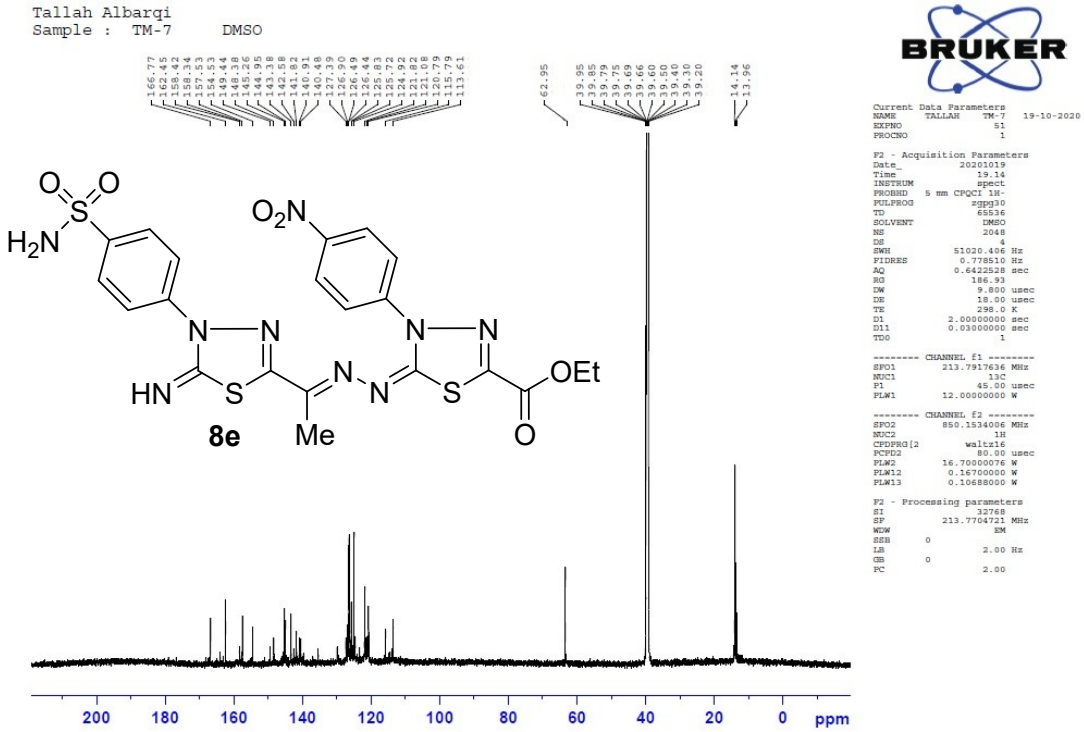

Figure S38. ^{13}C-NMR spectrum of compound **8e**.

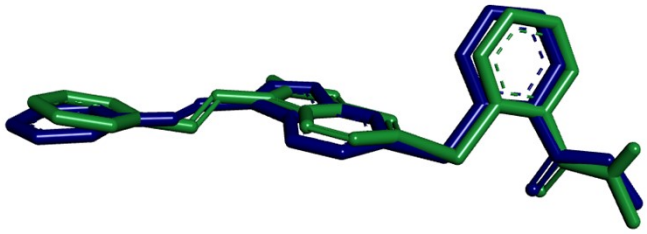

Figure S39. The superimposed conformations of the co-crystallized ligand **AXI** and its docked pose during molecular docking validation.

Supplementary Table S1. The MDS results of derivatives **2, 5a-c**, **8a-c**, and **8e** using VEGFR-2 (PDB: 4AG8)

Compound	Binding energy (Kcal/mol)	Type of interaction	Interacting residues	Distance (A^0)	Compound	Binding energy (Kcal/mol)	Type of interaction	Interacting residues	Distance (A^0)
AXI	-9.90	H-Acceptor	Asp1046	2.14	**8a**	-8.00	H-Acceptor	Lys868	2.59
		H-Donor	Glu917	3.05			H-Acceptor	Cys919	2.24
		H-Donor	Glu885	1.84			H-Acceptor	Asp1046	1.86
		Pi-Sigma	Leu840	2.60			Pi-Pi T-shaped	Phe1047	5.04
		Pi-Sigma	Lys868	2.63			Pi-Alkyl	Leu840	4.27
		Pi-Pi T-shaped	Phe1047	5.00			Pi-Alkyl	Ala866	5.12
		Pi-Alkyl	Val848	4.46			Pi-Alkyl	Leu1035	4.60
		Pi-Alkyl	Val848	5.46			Pi-Alkyl	Val848	4.43
		Pi-Alkyl	Ala866	4.23			Pi-Alkyl	Ala866	4.50
		Pi-Alkyl	Ala866	3.51			Pi-Alkyl	Val899	5.25
		Pi-Alkyl	Leu1035	4.67			Pi-Alkyl	Leu1035	5.00
		Pi-Alkyl	Leu1035	4.38			Pi-Alkyl	Cys1045	4.41
		Pi-Alkyl	Cys1045	4.76					
		Pi-Alkyl	Cys919	4.69	**8b**	-7.80	H-Acceptor	Lys868	2.41
		Pi-Alkyl	Val916	3.97			H-Donor	Glu917	2.21
							C-H Bond	Lys868	2.50
2	-8.00	H-Donor	Asp1046	2.10			H-Acceptor	Phe921	3.02
		C-H Bond	Gly922	2.55			Pi-Alkyl	Phe921	4.95
		Pi-Sigma	Phe1047	3.79			Pi-Alkyl	Leu840	4.94
		Pi-Pi T-shaped	Phe1047	5.47			Pi-Alkyl	Val848	5.42
		Alkyl	Leu889	4.96			Pi-Alkyl	Ala866	4.38
		Pi-Alkyl	Val848	4.97			Pi-Alkyl	Cys919	5.33
		Pi-Alkyl	Ala866	4.01			Pi-Alkyl	Leu1035	4.20
		Pi-Alkyl	Val899	5.45			Pi-Alkyl	Val848	4.61
		Pi-Alkyl	Leu1035	4.30			Pi-Alkyl	Ala866	4.44
		Pi-Alkyl	Cys1045	4.99			Pi-Alkyl	Val899	4.72
		Pi-Alkyl	Leu840	3.79			Pi-Alkyl	Val916	4.58
		Pi-Alkyl	Leu1035	5.32			Pi-Alkyl	Cys1045	4.66
							Pi-Alkyl	Leu840	5.29
5a	-8.40	H-Acceptor	Lys868	2.42					

		H-Acceptor	Cys919	2.70	**8c**	-7.90	H-Acceptor	Lys868	2.30
		H-Donor	Glu917	2.18			H-Donor	Glu917	2.20
		H-Donor	Glu885	2.14			H-Donor	Glu885	2.16
		Pi-Alkyl	Leu840	5.12			Amide-Pi Stacked	Phe921:C,O;Gly922:N	4.93
		Pi-Alkyl	Ala866	4.42			Pi-Alkyl	Phe918	5.07
		Pi-Alkyl	Cys919	5.16			Pi-Alkyl	Leu840	4.96
		Pi-Alkyl	Leu1035	4.04			Pi-Alkyl	Val848	5.43
		Pi-Alkyl	Val848	4.73			Pi-Alkyl	Ala866	4.37
		Pi-Alkyl	Ala866	4.46			Pi-Alkyl	Cys919	5.30
		Pi-Alkyl	Val899	4.59			Pi-Alkyl	Leu1035	4.19
		Pi-Alkyl	Val916	4.52			Pi-Alkyl	Val848	4.60
		Pi-Alkyl	Cys1045	4.60			Pi-Alkyl	Ala866	4.43
		Pi-Alkyl	Leu840	5.00			Pi-Alkyl	Val899	4.73
							Pi-Alkyl	Val916	4.57
5b	-7.90	H-Acceptor	Lys868	2.29			Pi-Alkyl	Cys1045	4.68
		H-Donor	Glu917	2.43			Pi-Alkyl	Leu840	5.27
		H-Donor	Glu885	2.23					
		Amide-Pi Stacked	Phe921:C,O;Gly922N	5.08	**8e**	-8.40	H-Acceptor	Lys868	2.31
		Pi-Alkyl	Leu840	5.02			H-Acceptor	Asn923	2.82
		Pi-Alkyl	Leu840	5.10			H-Donor	Glu917	2.34
		Pi-Alkyl	Val848	5.34			Pi-Alkyl	Leu840	5.04
		Pi-Alkyl	Val848	4.67			Pi-Alkyl	Leu840	5.20
		Pi-Alkyl	Ala866	4.32			Pi-Alkyl	Val848	5.02
		Pi-Alkyl	Ala866	4.42			Pi-Alkyl	Ala866	4.03
		Pi-Alkyl	Cys919	5.36			Pi-Alkyl	Leu1035	4.34
		Pi-Alkyl	Leu1035	4.19			Pi-Alkyl	Val848	4.92
		Pi-Alkyl	Val899	4.65			Pi-Alkyl	Ala866	4.78
		Pi-Alkyl	Val916	4.42			Pi-Alkyl	Val899	4.50
		Pi-Alkyl	Cys1045	4.73			Pi-Alkyl	Val916	4.23
							Pi-Alkyl	Cys1045	4.68
5c	-7.90	H-Acceptor	Lys868	2.53					
		H-Acceptor	Asn923	2.78					
		H-Acceptor	Asp1046	2.04					
		H-Acceptor	Leu840	2.86					
		Pi-Pi T-shaped	Phe1047	5.00					

Supplementary Table 2. The physicochemical and pharmacokinetics properties of **5a-b**, **8a-c** and **8e** as obtained from SwissADME.

Compounds	5a	5b	8a	8b	8c	8e
Physicochemical properties						
Molecular weight in g/mole	532.6	549.1	558.7	579.1	623.5	589.6
Number of heavy atoms	35.0	35.0	37.0	37.0	37.0	39.0
Number of rotatable bonds	6.0	6.0	8.0	8.0	8.0	9.0
Number of H-bond acceptors	10.0	9.0	10.0	10.0	10.0	12.0
Number of H-bond donors	2.0	2.0	2.0	2.0	2.0	2.0
Molar refractivity	128.5	133.6	139.4	139.5	142.2	143.3
TPSA ($Å^2$)	226.3	226.3	235.5	235.5	235.5	281.4
Log $P_{o/w}$	3.5	3.6	3.2	3.5	3.7	2.8
Pharmacokinetics properties						
GI absorption	Low	Low	Low	Low	Low	Low
BBB permeant	No	No	No	No	No	No
P-glycoprotein substrate	Yes	Yes	No	No	No	Yes
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
Skin Permeation log $Kp_{(cm/s)}$	-2.7	-2.7	-6.7	-6.7	-6.9	-7.3
Drug Likeness						
Lipinski #violations	1	1	1	1	1	2
Veber #violations	1	1	1	1	1	1
Egan #violations	1	1	1	1	1	1
Bioavailability Score	0.17	0.17	0.17	0.17	0.17	0.17