Supplementary Data

Tailoring of novel morpholine-sulphonamide linked thiazole moiety as dual targeting DHFR/DNA gyrase inhibitors: Synthesis, antimicrobial, antibiofilm activities and DFT with molecular modelling studies

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1. Materials and Instrumental techniques

Melting points were defined on a SMP50 Digital APP (Bibby Scientific, Staffordshire, UK) 120/230V, in open capillaries and are uncorrected. The mid-IR spectra (KBr, ν/cm^{-1}) were recorded on the CARY 630 FT-IR spectrophotometer (Agilent, Santa Clara, CA, USA). The NMR spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker EX-400 MHz (^{1}H: 300/500 MHz, ^{13}C: 75/125 MHz) spectrometer (Bruker, USA) at room temperature. Tetramethylsilane (TMS) is used for internal calibration (^{1}H NMR and ^{13}C NMR: 0.00 ppm). The chemical shifts were reported in parts per million (ppm) on the δ scale and relative to residual solvent peaks ($CDCl_3$: ^{1}H: 7.26 ppm, ^{13}C: 77.5 ppm, DMSO-d_6: ^{1}H: 2.50 ppm, ^{13}C: 39.5 ppm). Coupling constants (*J*) are reported in Hz with the following abbreviations used to indicate splitting: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal.

Electron ionization mass spectrometry (EI-MS) spectra were attained through the Thermo Scientific™ ISQ™ LT Single Quadrupole GC-MS system using Xcalibur data acquisition software (Shimadzu, Kyoto, Japan) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, Egypt). For the chemical synthesis, starting materials and reagents were obtained from commercial sources particularly Acros Organics ™ (Geel, Belgium) and used without further purification, unless otherwise indicated. Solvents were obtained from Thermo Fisher Scientific ™ and employed without additional purification. For biological screening, clinical Specimens (urine, abscess, tooth, sputum, tonsils, wound, and throat swabs) were collected from Damanhur National Medical Institute (DNMI), Egypt. Chemical syntheses were done using miniscale reaction apparatus, and their procedures were not optimized. All the reactions were implemented utilizing oven-dried glassware. The purity of the synthesized compounds was investigated by TLC, performed on Merck precoated silica gel 60 F_{254} aluminum sheets with solvent mixture of DCM-MeOH (99-1) as eluent system. Spots were visualized under UV illumination at 254 nm. Compounds names are derived from ChemOffice-v2020 and are not necessarily identical with the IUPAC nomenclature. Schemes were produced by the same version of ChemOffice. IR data were plotted by OriginPro-v2021. NMR spectra were delineated *via* MestReNova-v14.2.1.

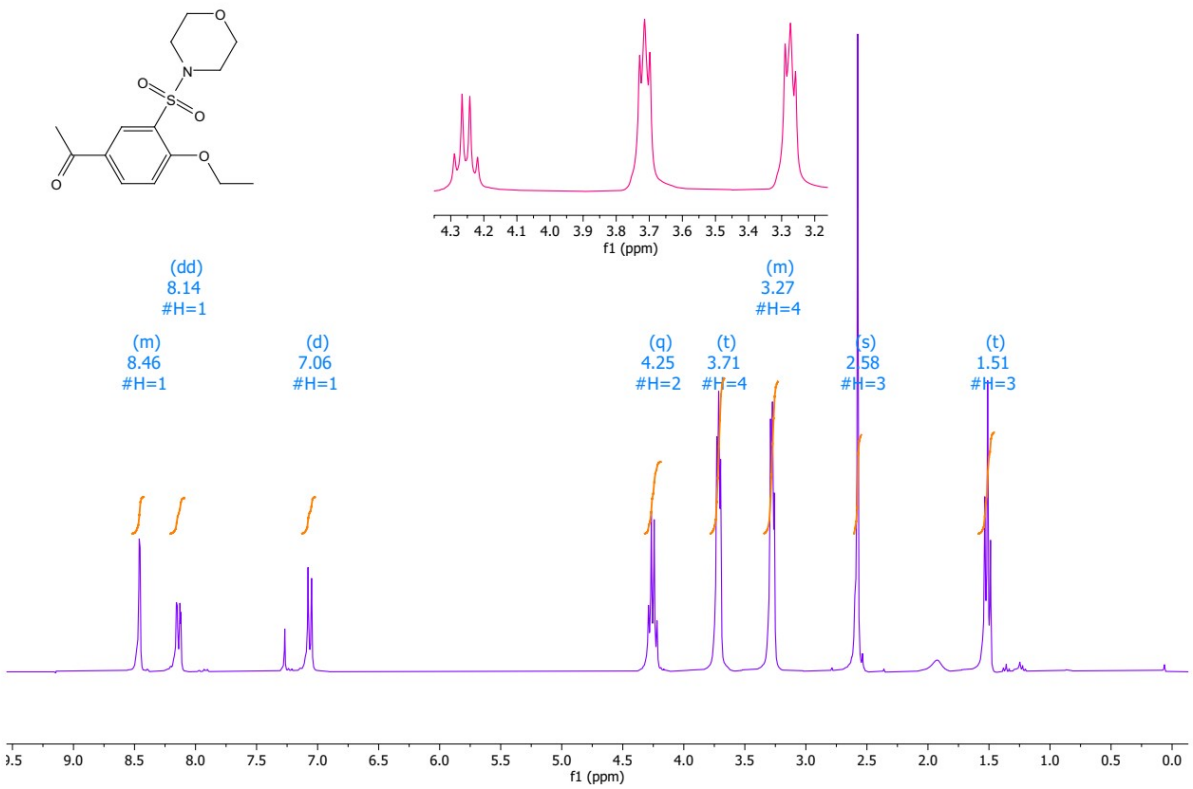

Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) of **2**.

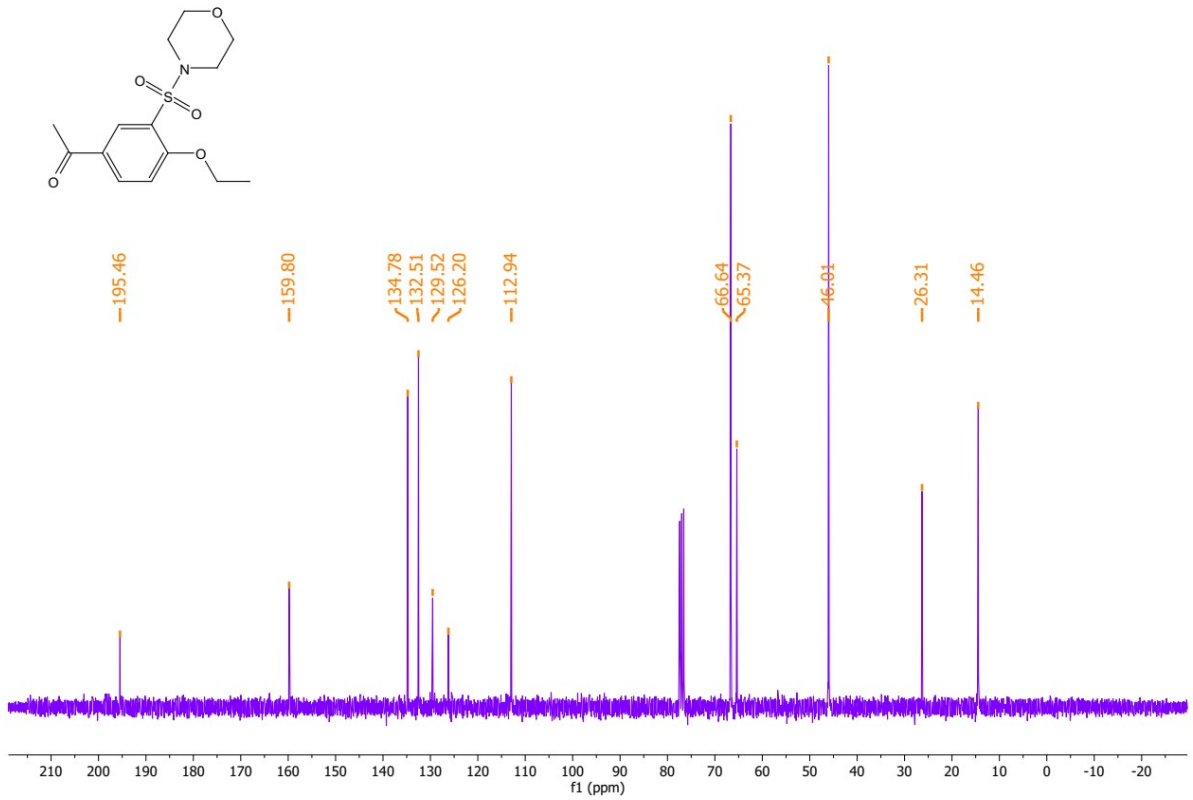

Figure S2. ^{13}C NMR (75 MHz, $CDCl_3$) of **2**.

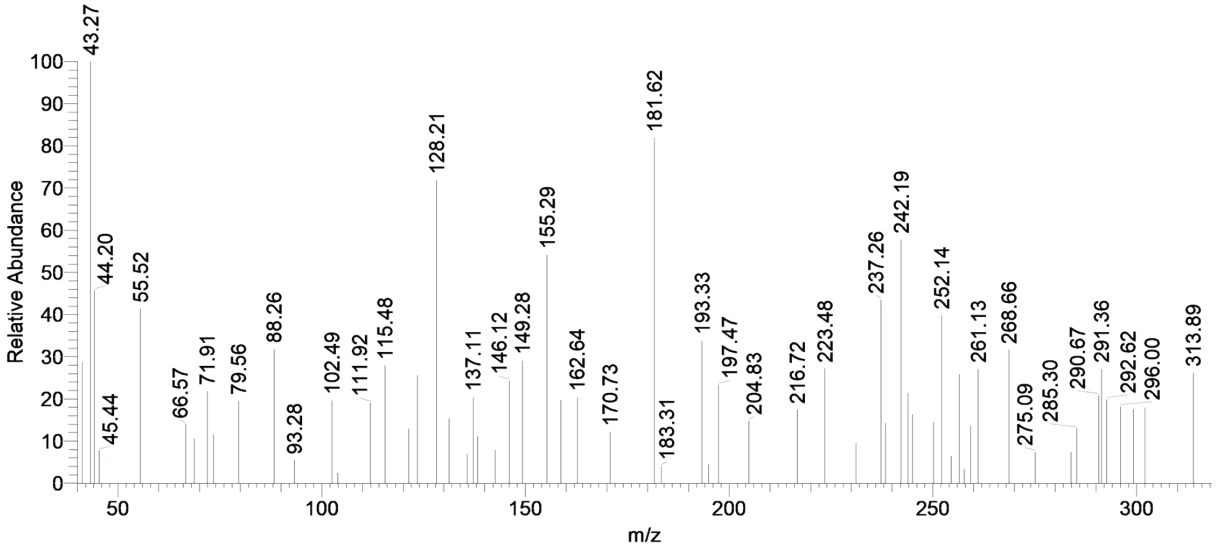

Figure S3. EI-MS of **2**.

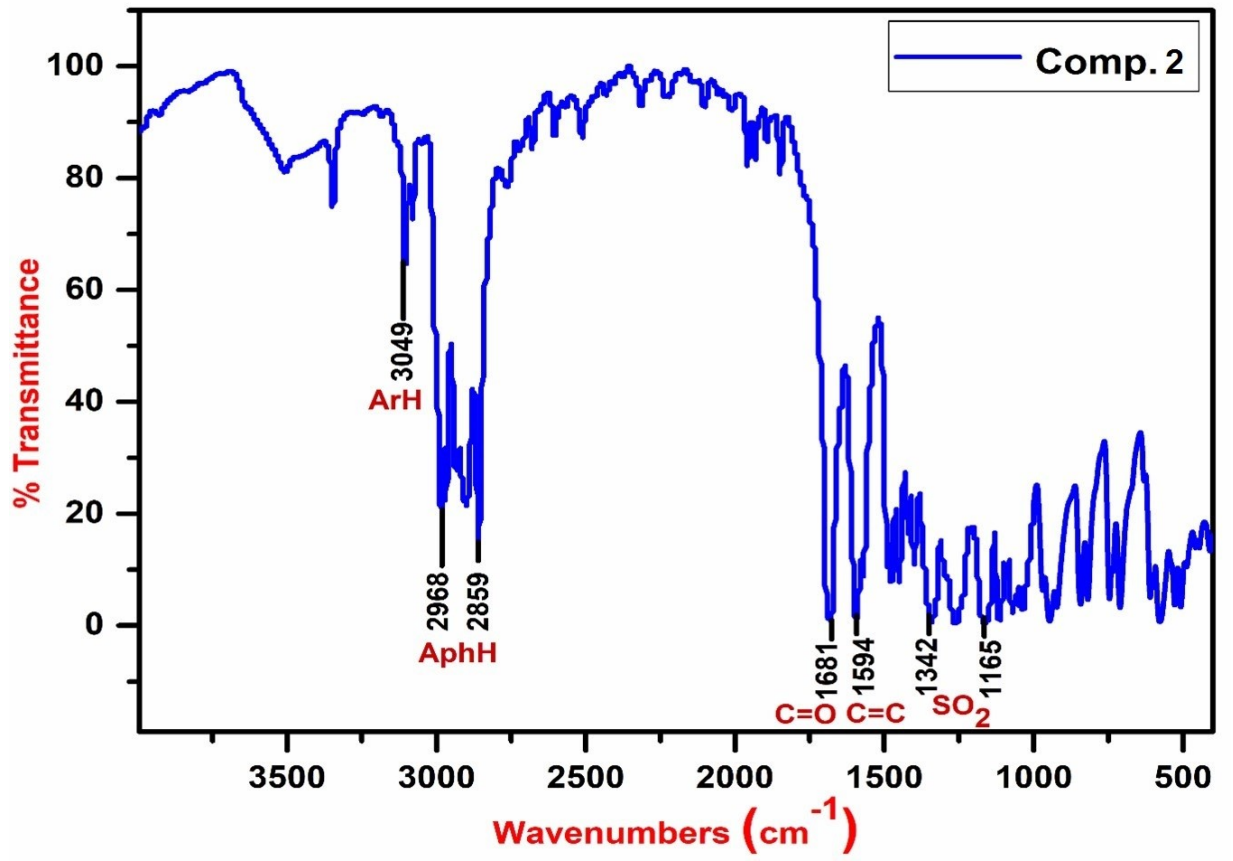

Figure S4. FT-IR of **2**.

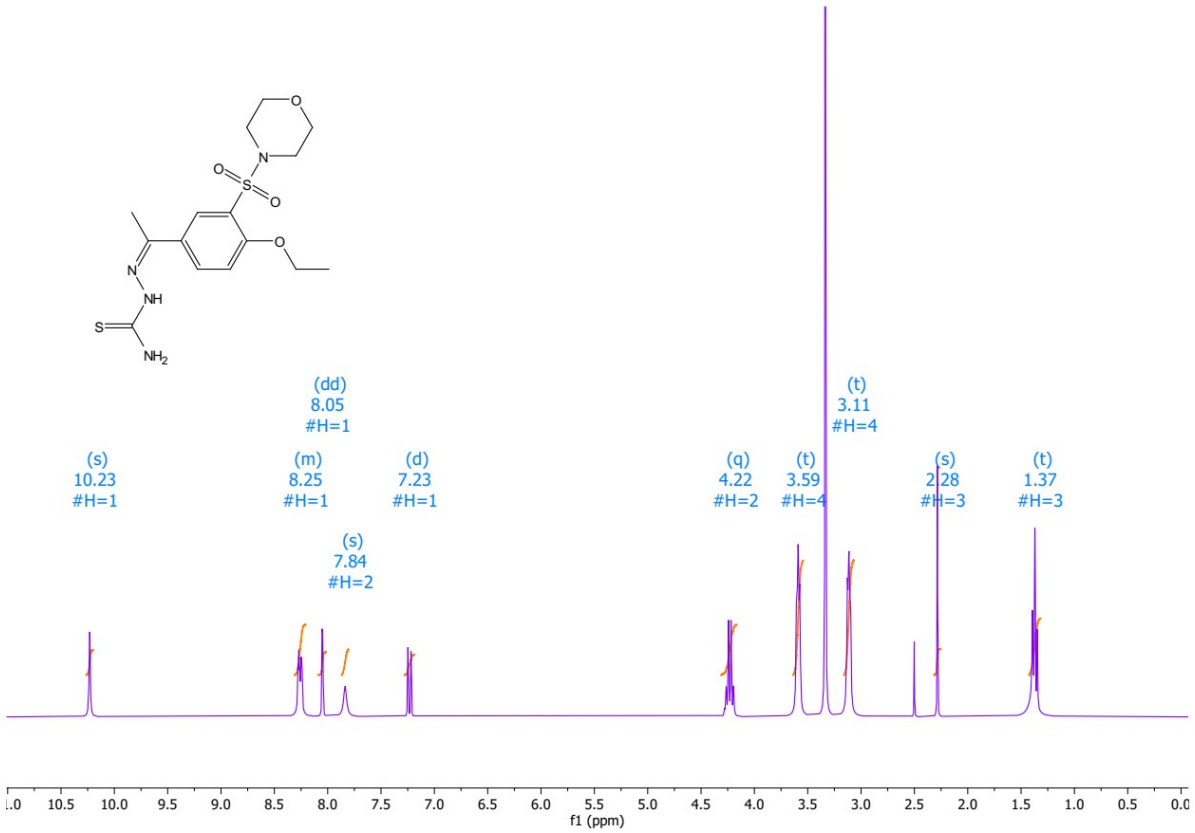

Figure S5. ^{1}H NMR (300 MHz, DMSO-d_6) of **3**.

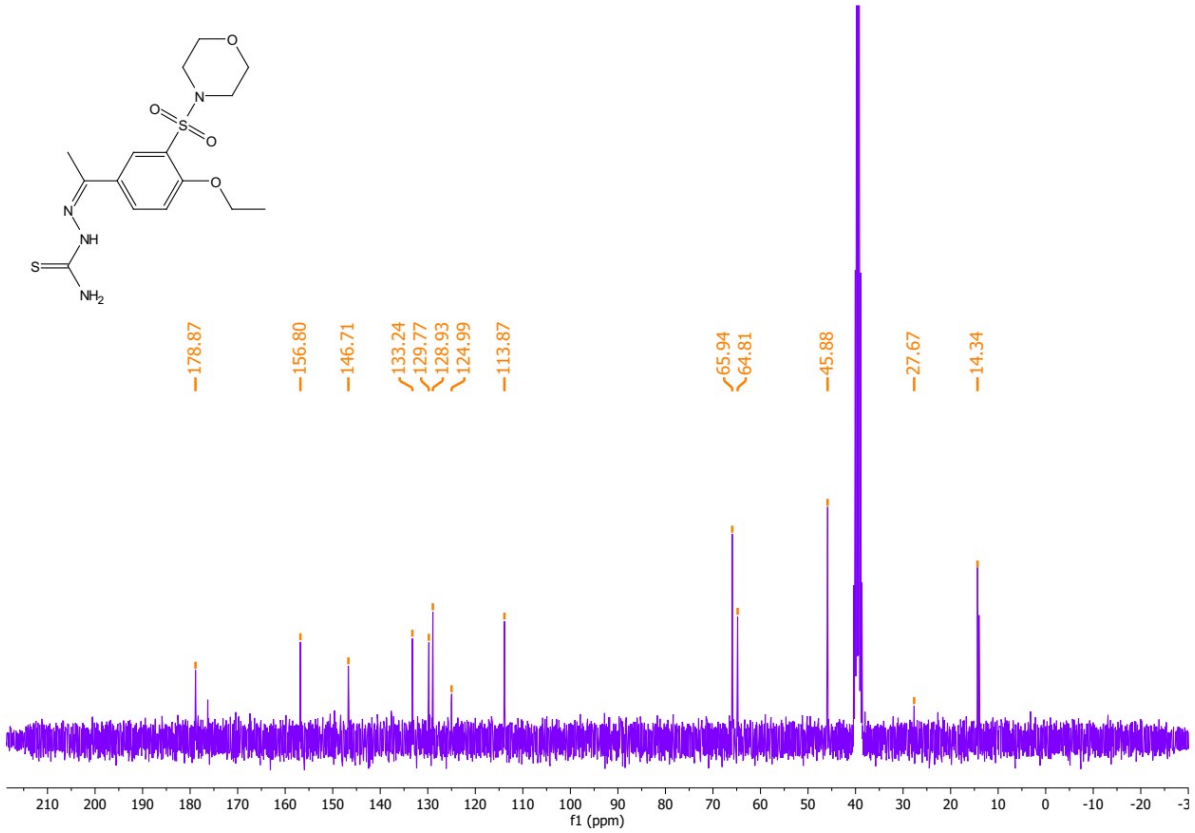

Figure S6. ^{13}C NMR (75 MHz, DMSO-d_6) of **3**.

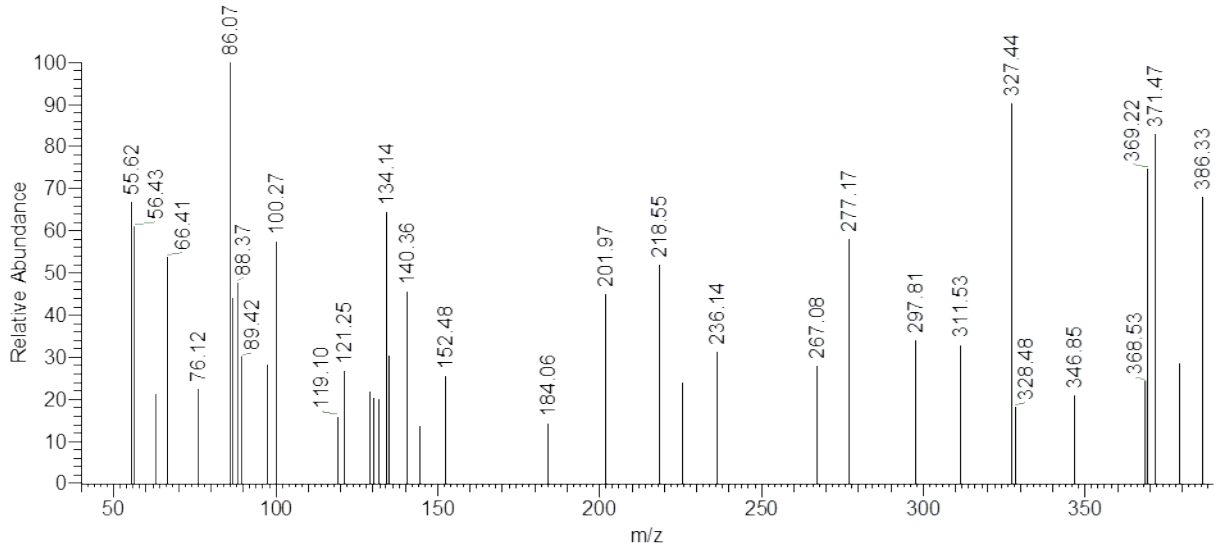

Figure S7. EI-MS of **3**.

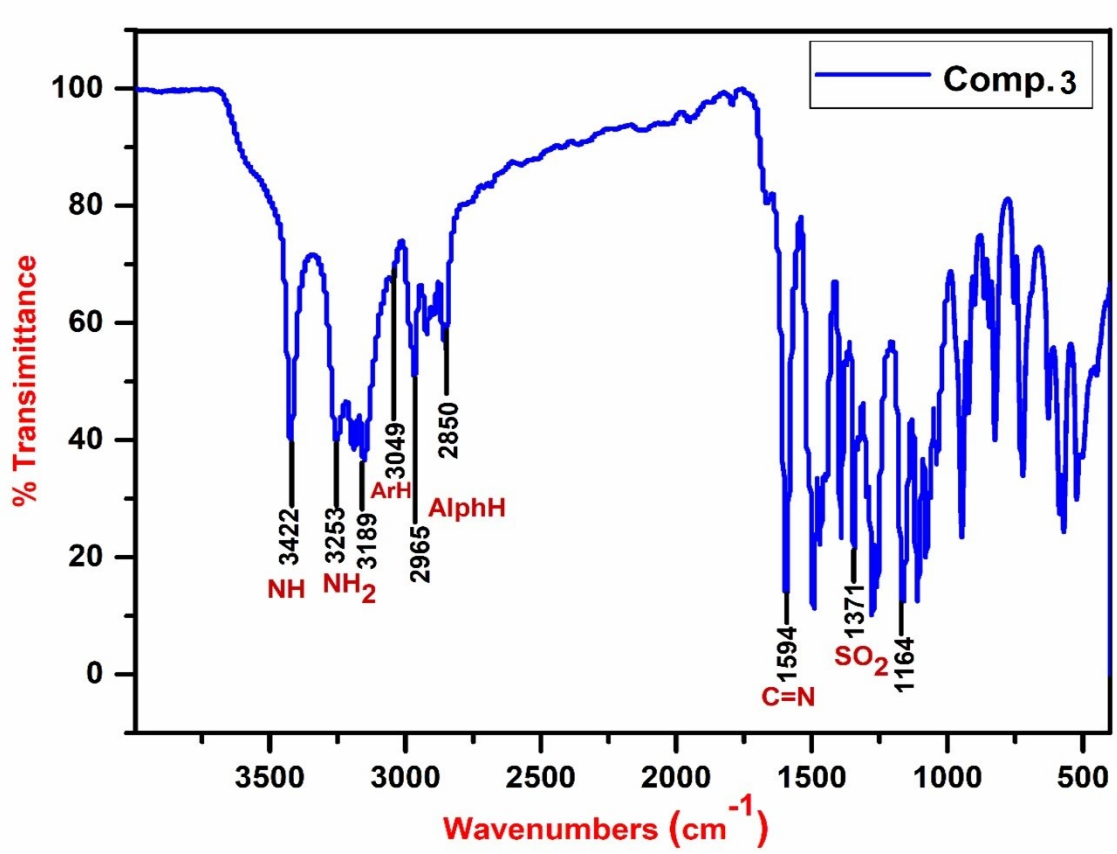

Figure S8. FT-IR of **3**.

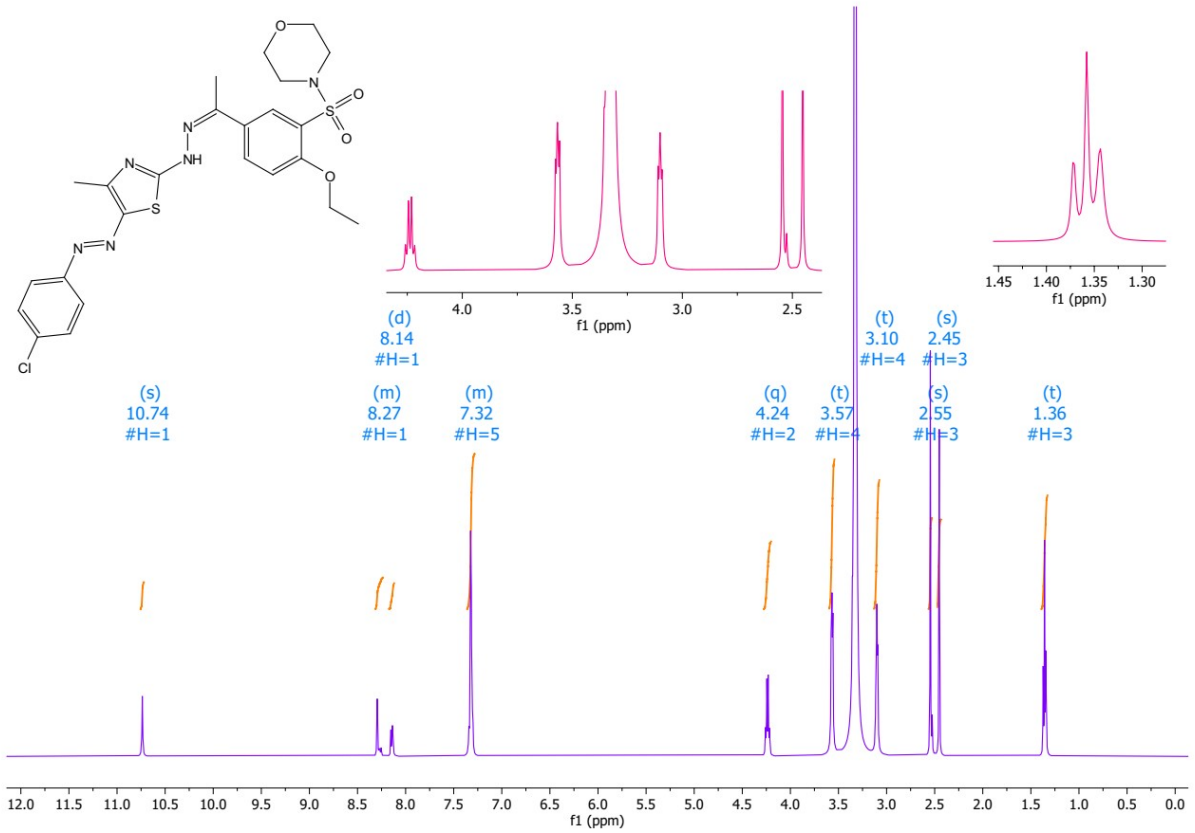

Figure S9. ^{1}H NMR (500 MHz, DMSO-d_6) of **4a**.

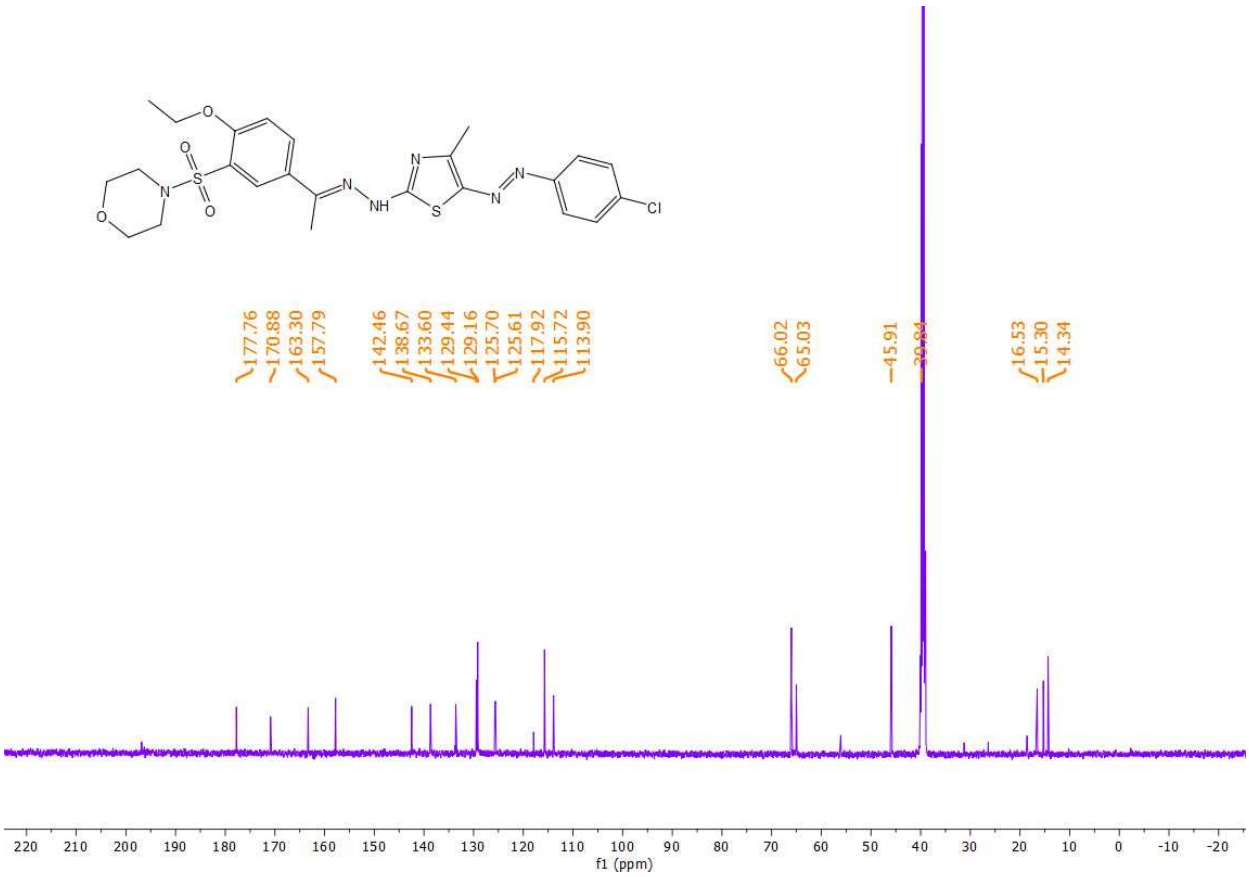

Figure S10. ^{13}C NMR (125 MHz, DMSO) of **4a**.

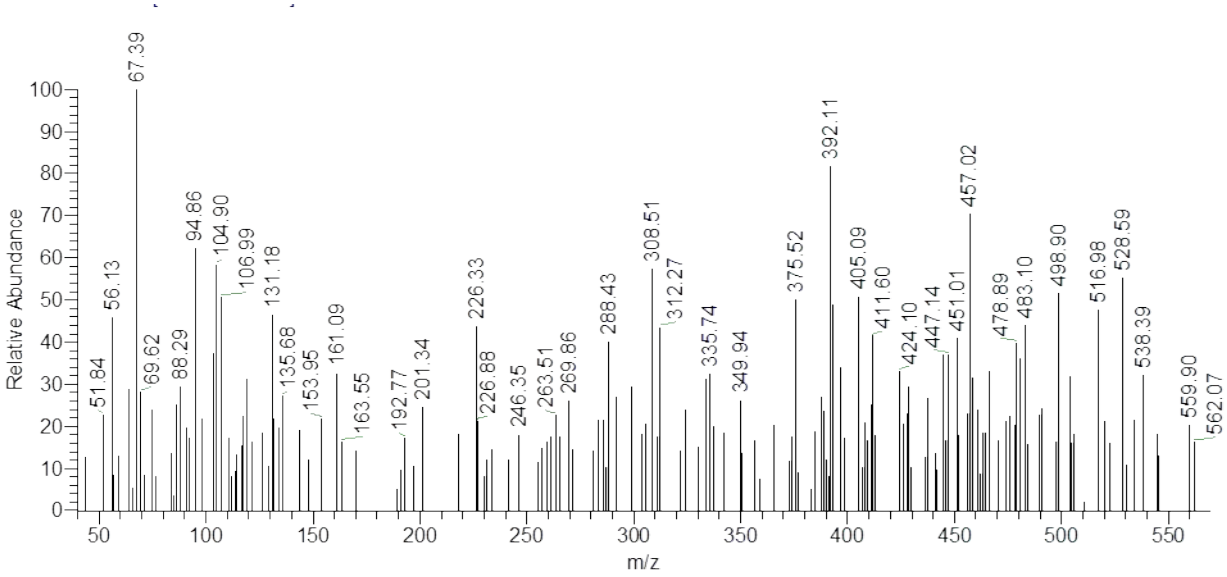

Figure S11. EI-MS of **4a**.

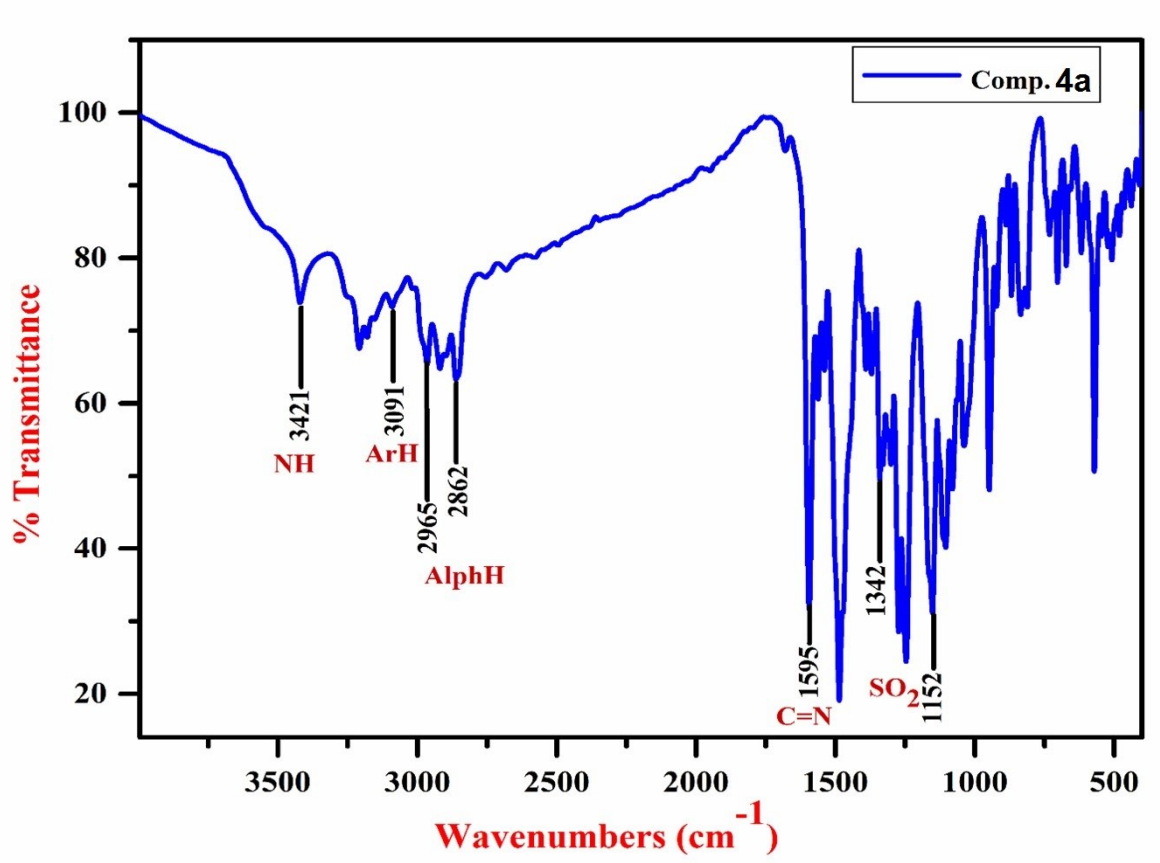

Figure S12. FT-IR of **4a**.

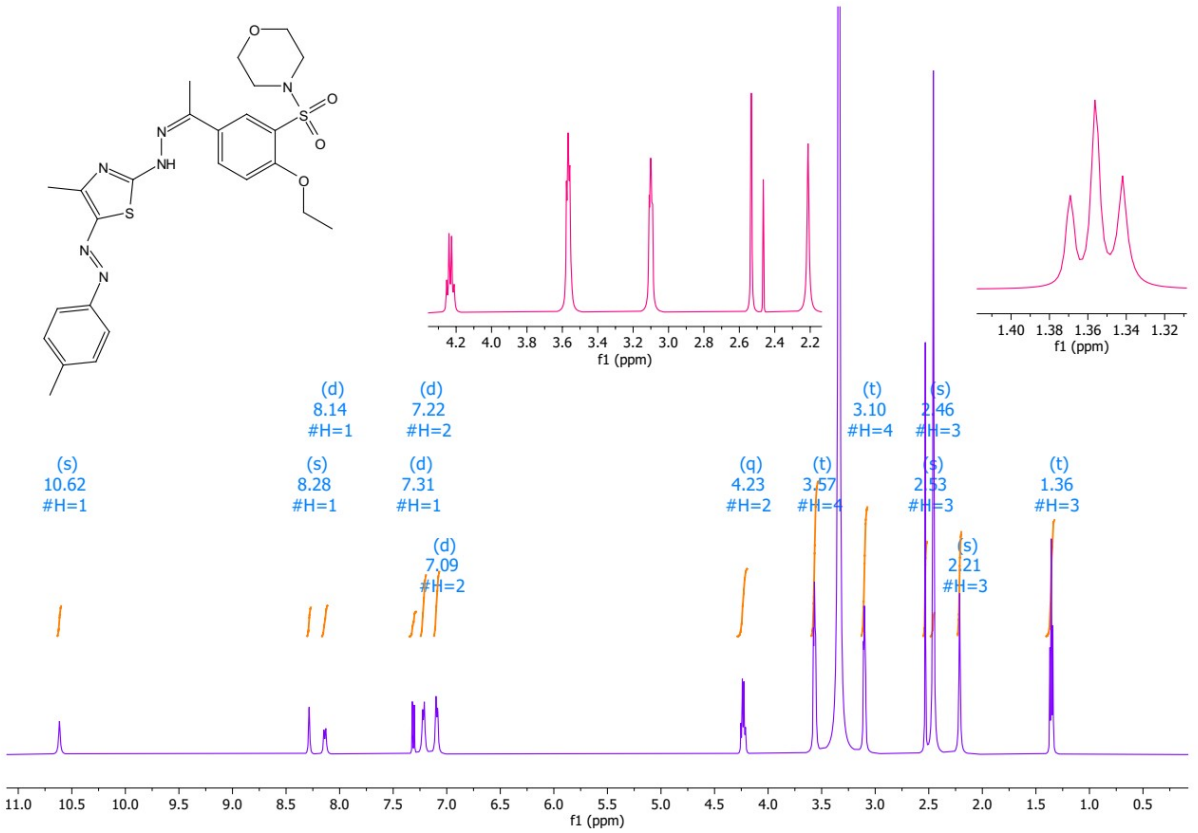

Figure S13. [1]H NMR (500 MHz, DMSO-d_6) of **4b**.

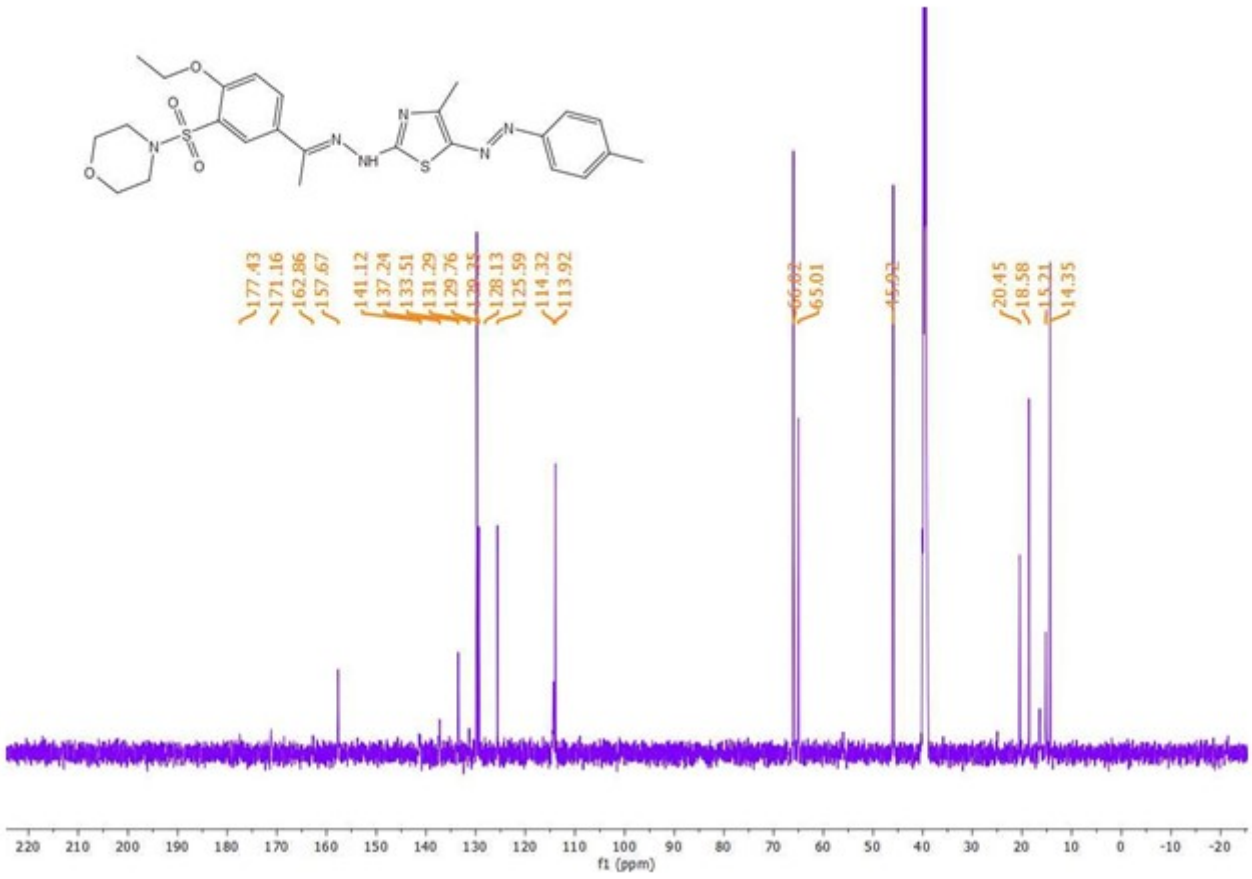

Figure S14. [13]C NMR (125 MHz, DMSO) of **4b**.

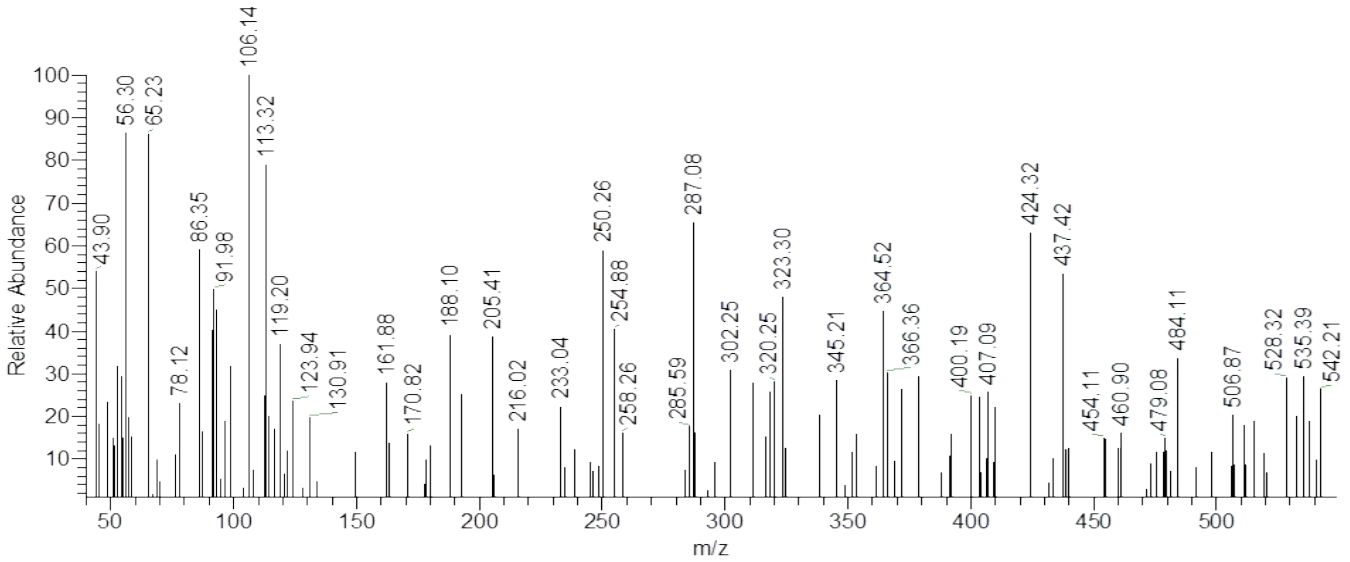

Figure S15. EI-MS of **4b**.

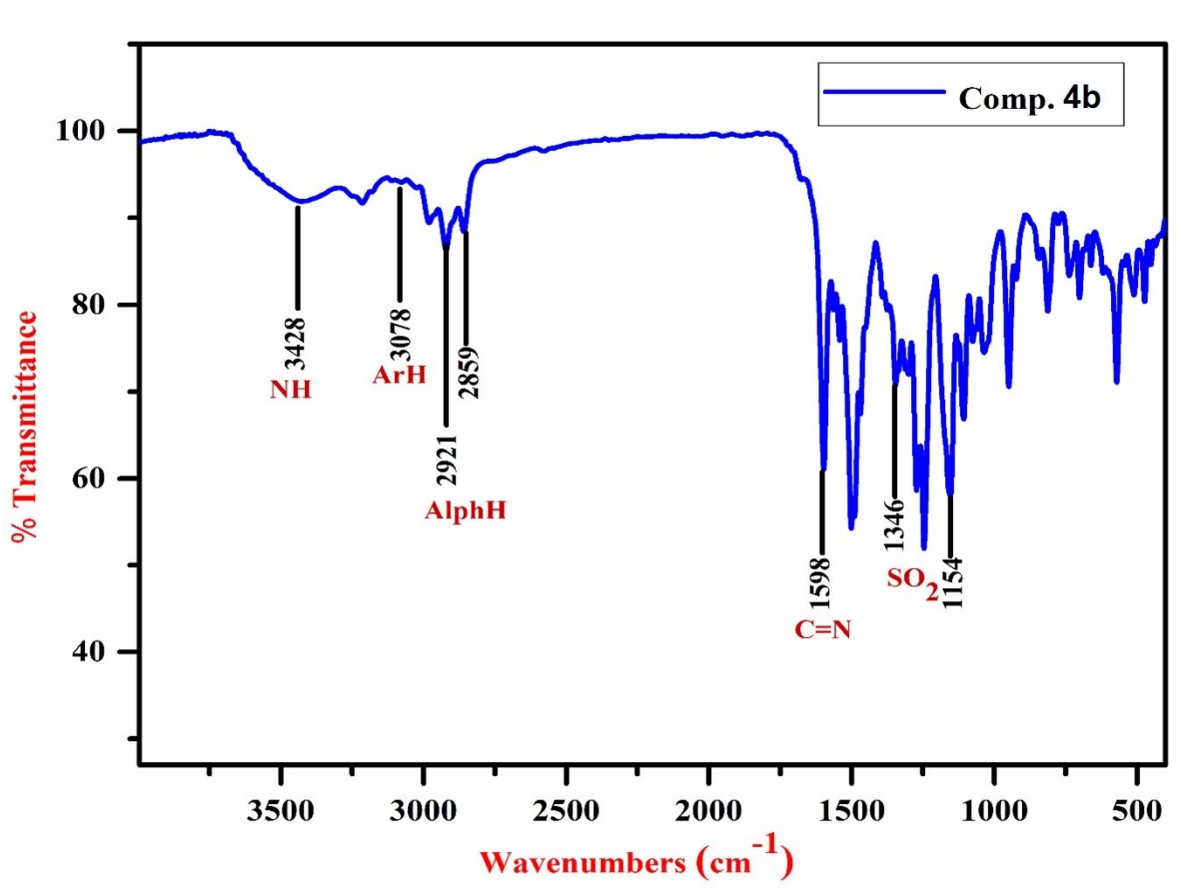

Figure S16. FT-IR of **4b**.

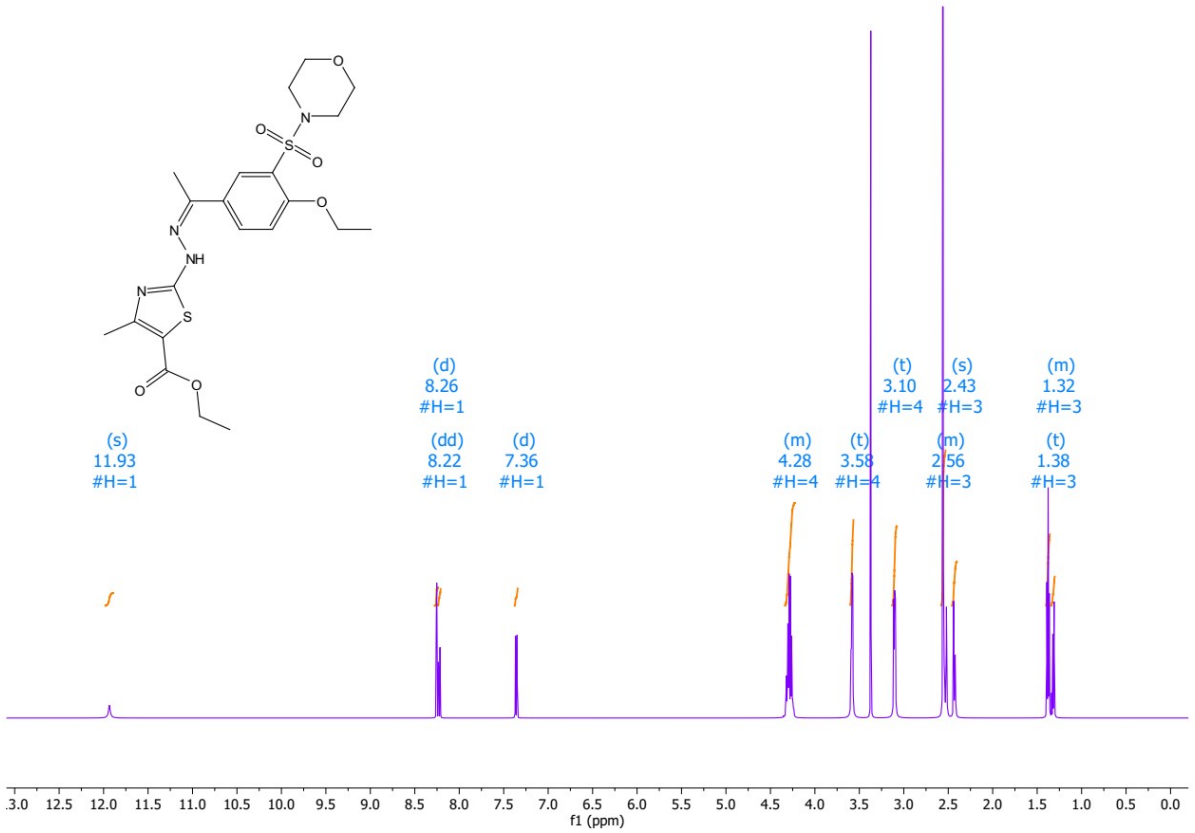

Figure S17. ^{1}H NMR (500 MHz, DMSO-d_6) of **5**.

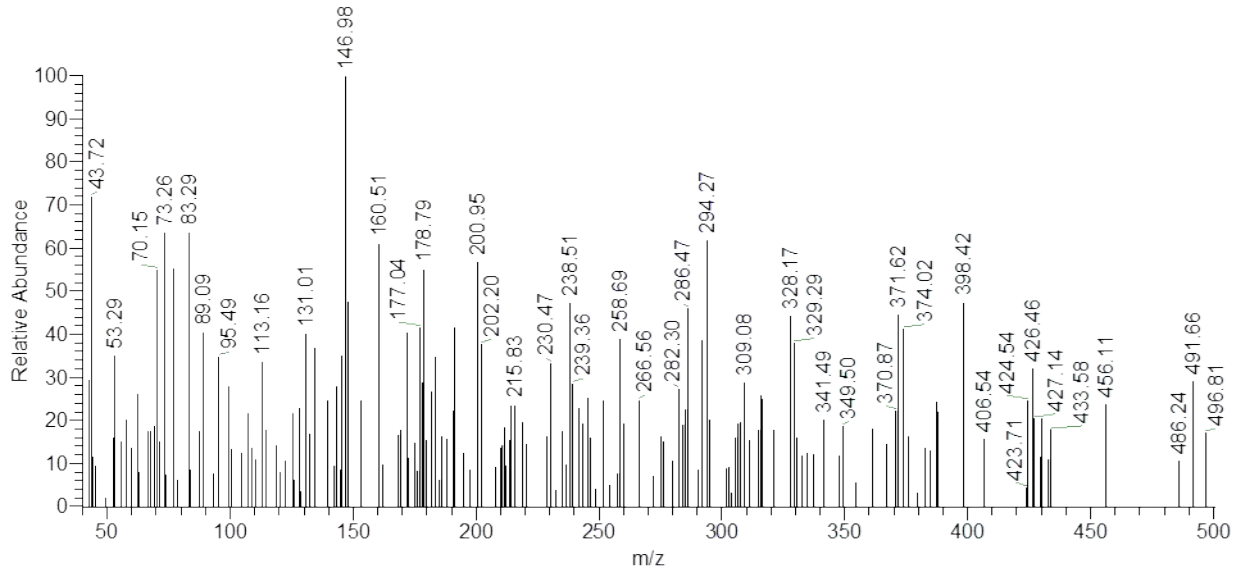

Figure S18. EI-MS of **5**.

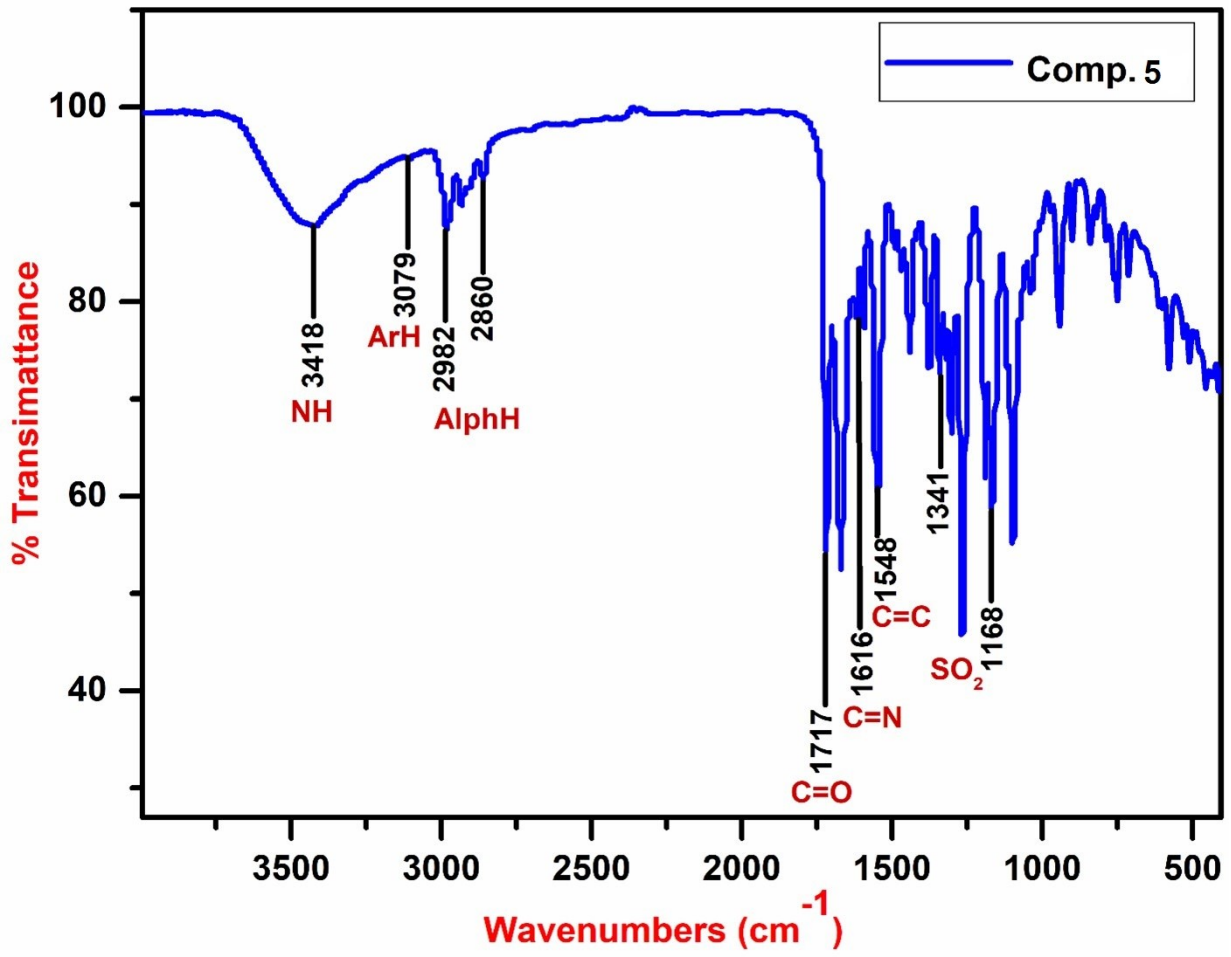

Figure S19. FT-IR of **5**.

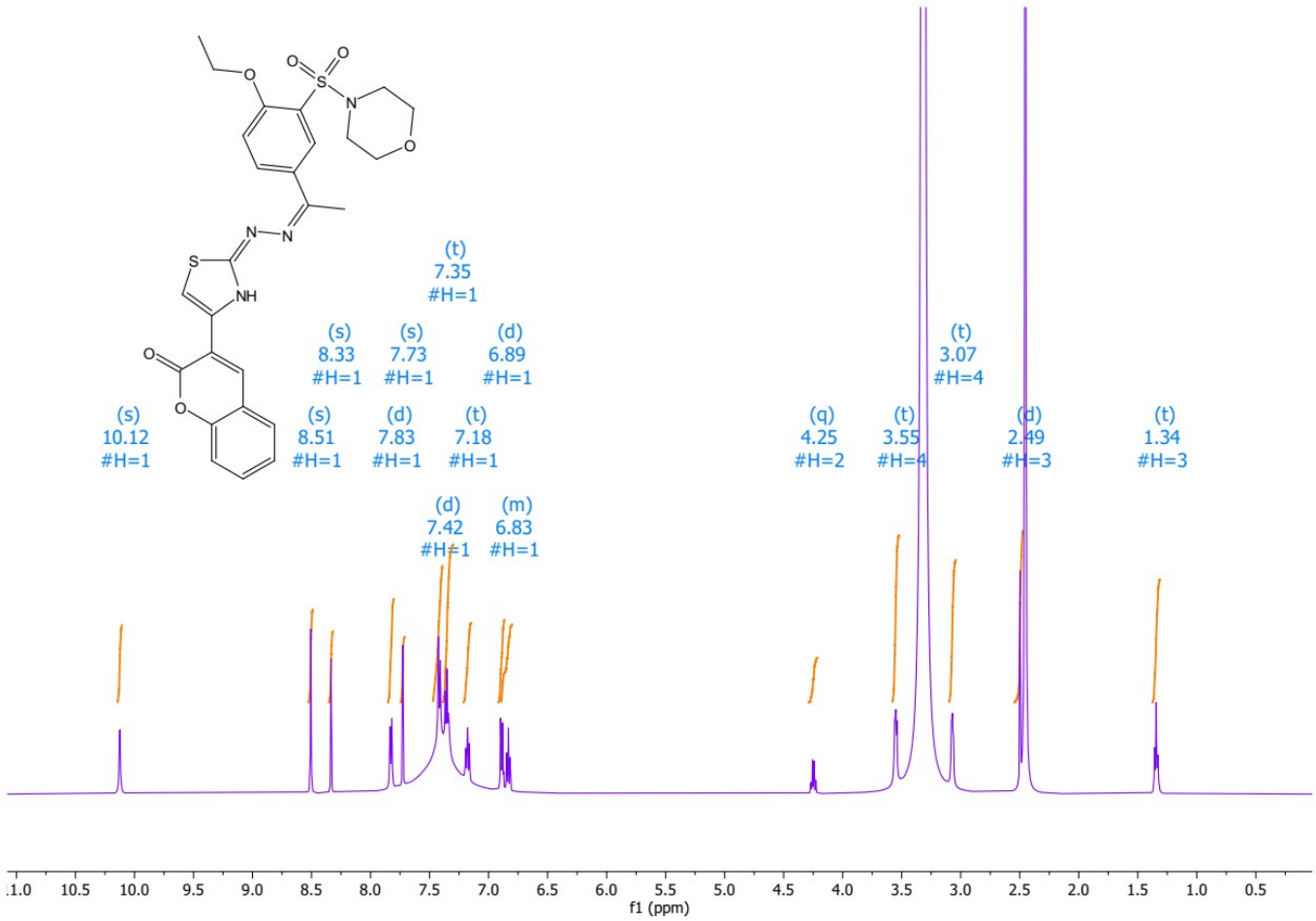

Figure S20. ^{1}H NMR (500 MHz, DMSO-d_6) of **6**.

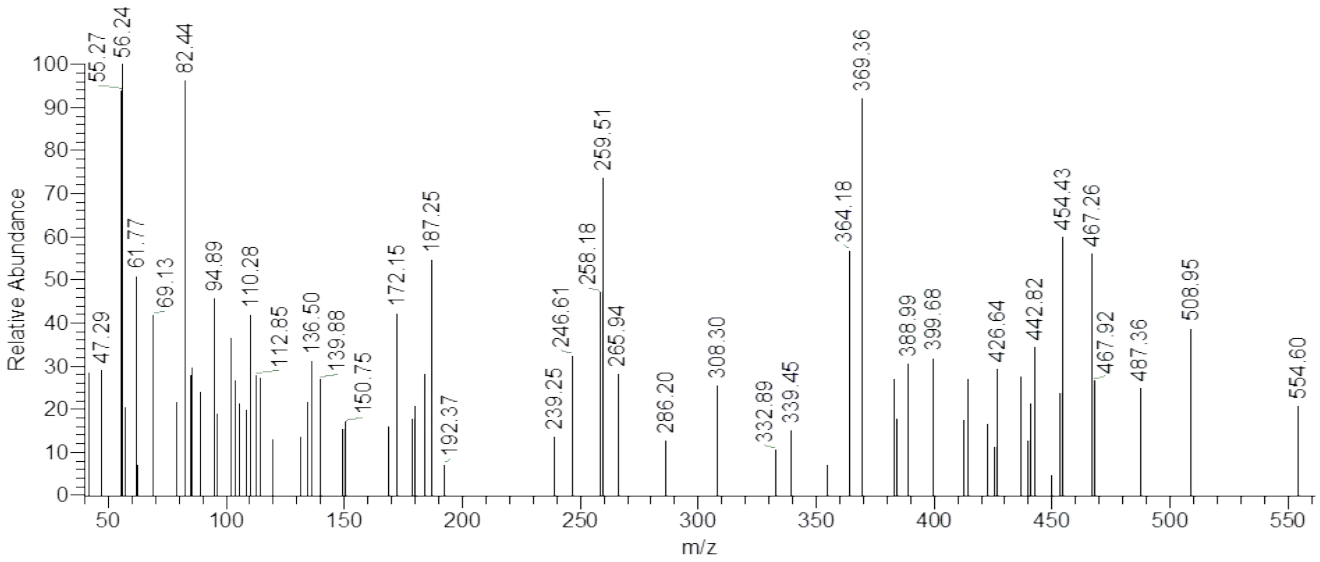

Figure S21. EI-MS of **6**.

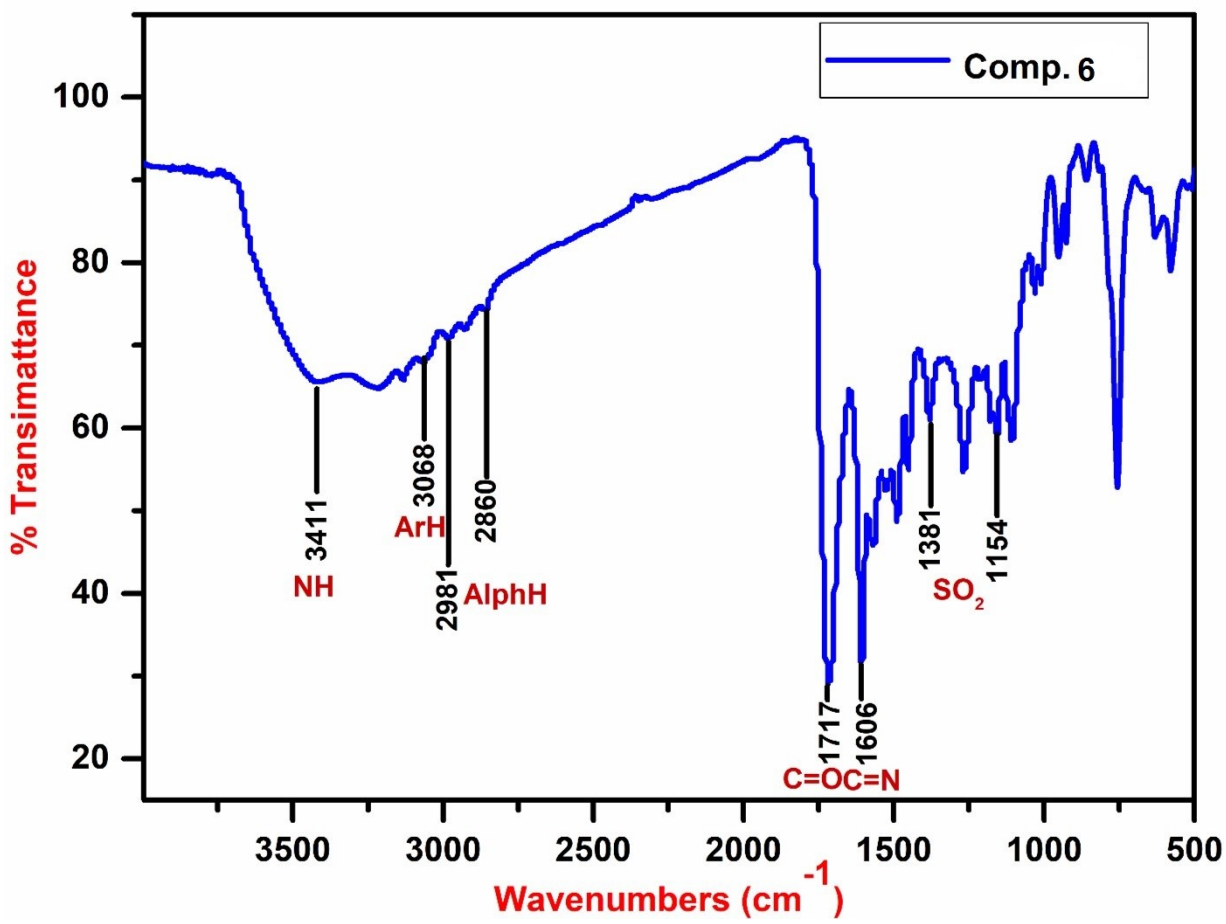

Figure S22. FT-IR of **6**.

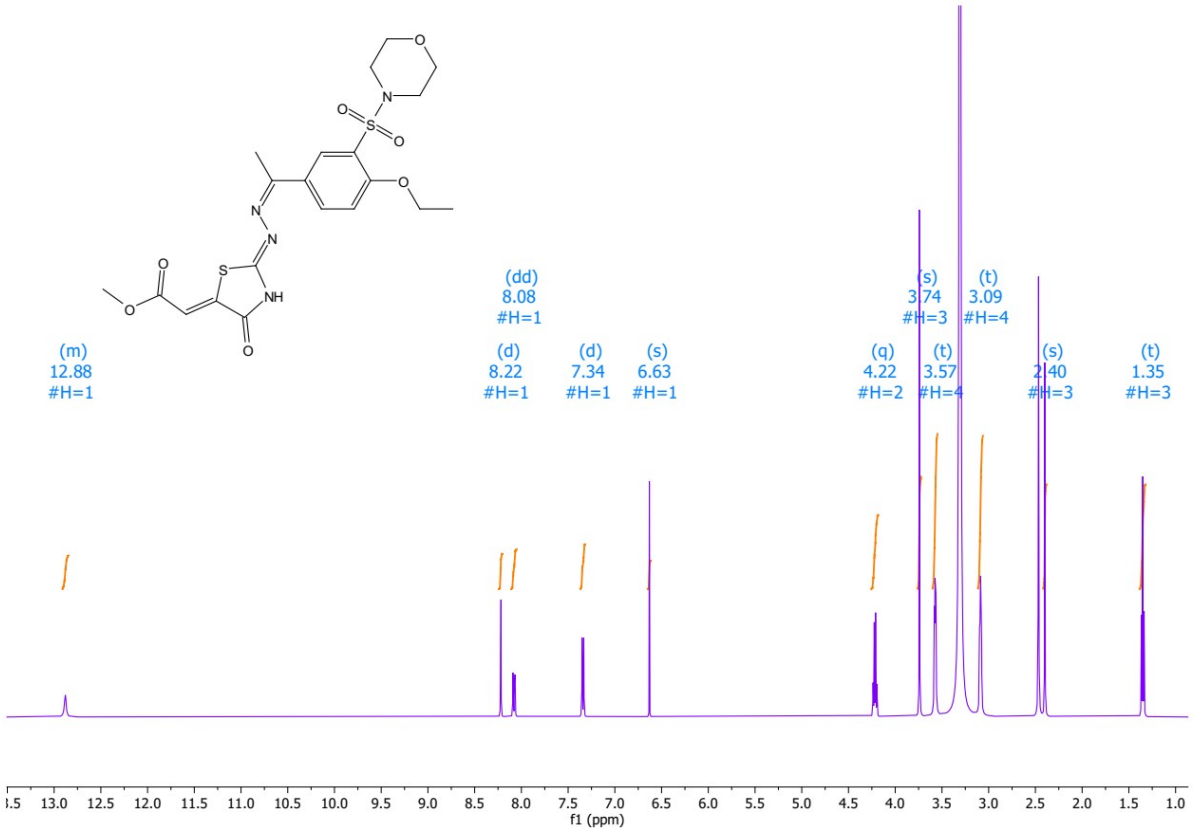

Figure S23. ^{1}H NMR (500 MHz, DMSO-d_6) of **7**.

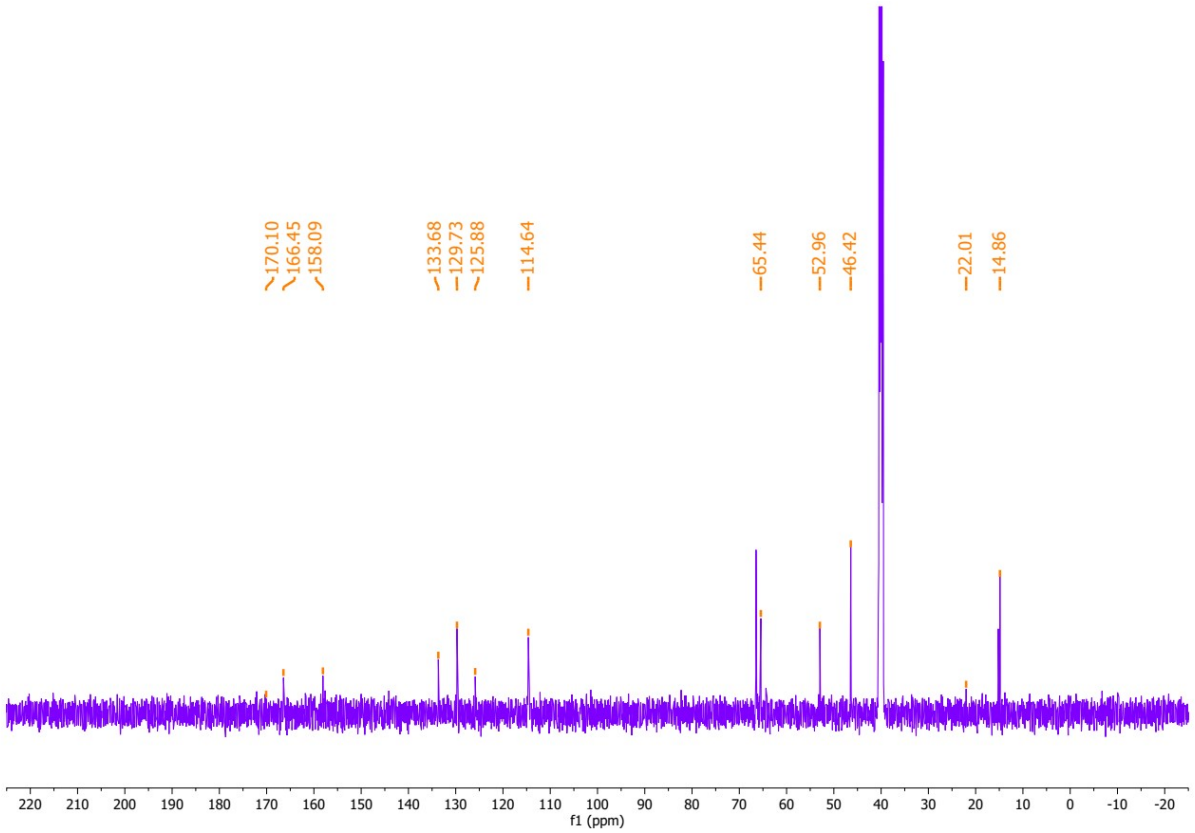

Figure S24. ^{13}C NMR (125 MHz, DMSO) of **7**.

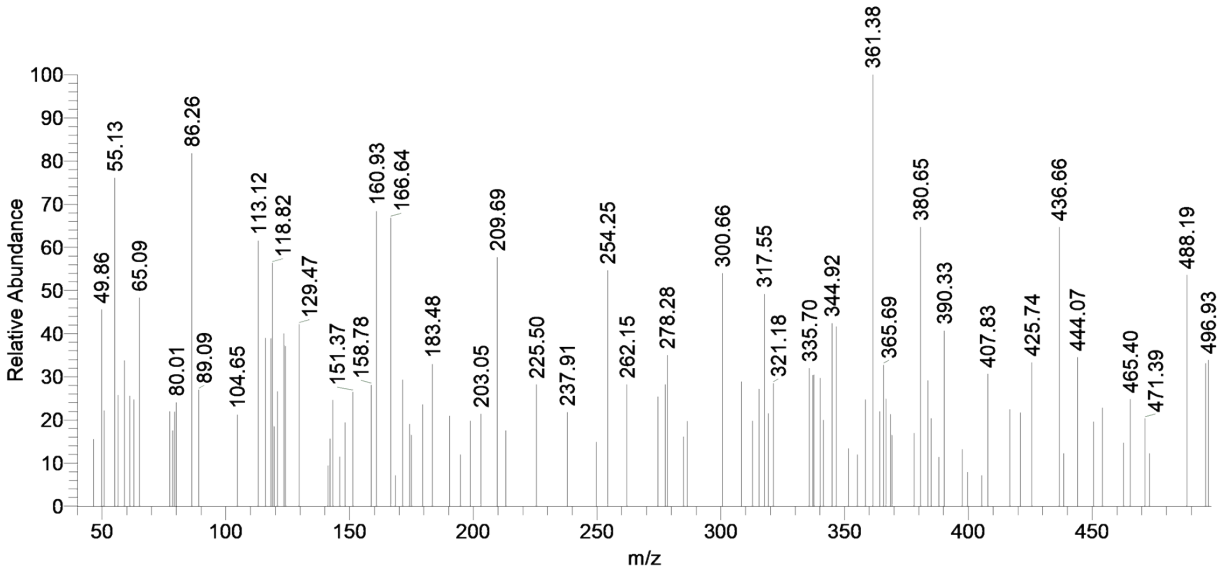

Figure S25. EI-MS of **7**.

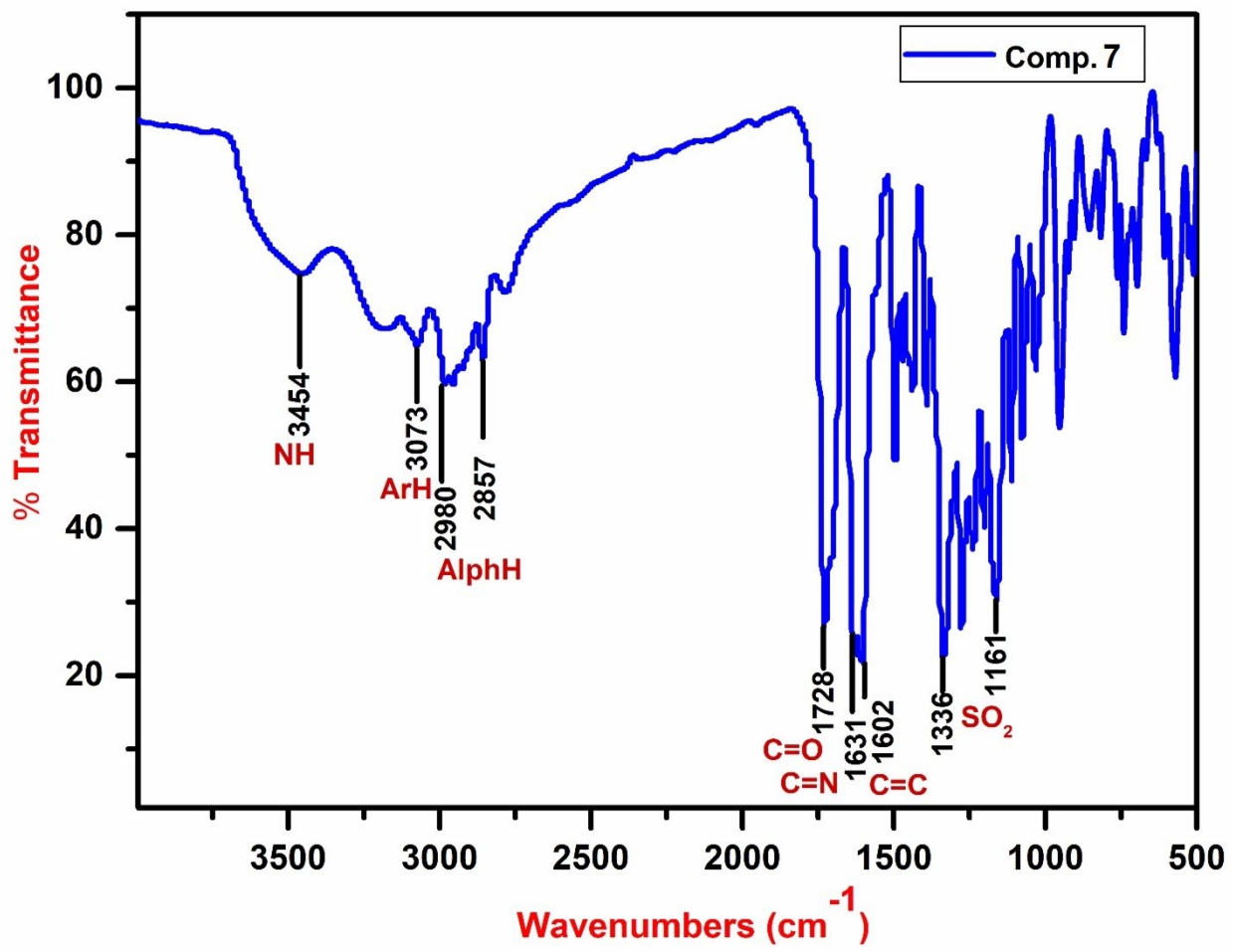

Figure S26. FT-IR of **7**.

Table S1. Physicochemical properties, lipophilicity, and drug-likeness nature of precursors **2**, **3**, and the target compounds **4a,b**–**7**.

Cpd.	Physicochemical properties					Lipophilicity		Drug-likeness (Accepted/Rejected)	
	MW (g/mol)	nRot[a] (≠)	nHA[b] (≠)	nHD[c] (≠)	TPSA[d] (Å²)	logP[e]	logD[f]	Lipinski rule[g]	Golden Triangle rule[h]
2	313.1	5	6	0	72.91	1.333	1.188	Accepted	Accepted
3	386.11	7	8	3	106.25	1.933	1.466	Accepted	Accepted
4a	562.12	9	10	1	117.84	5.585	4.625	*Rejected*	*Rejected*
4b	542.18	9	10	1	117.84	5.373	4.632	*Rejected*	*Rejected*
5	496.15	10	10	1	119.42	3.504	3.116	Accepted	Accepted
6	554.13	7	10	1	126.56	4.306	3.161	Accepted	*Rejected*
7	496.11	8	11	1	135.96	1.778	1.310	Accepted	Accepted

[a] nRot = no of Rotatable bonds; [b] nHA = Hydrogen Bond Acceptor; [c] nHD = Hydrogen Bond Donor; [d] TPSA = Topological polar surface area; [e] logP = The partition coefficient between *n*-octanol and water, optimal 0–3; [f] logD = logP at physiological pH 7.4, optimal 1–3; [g] Lipinski (RO5) criteria range are lipophilicity (logP) ≤ 5, MW ≤ 500, H-bond donors ≤ 5 and H-bond acceptors ≤ 10; [h] Golden Triangle criteria range are: 200 ≤ MW ≤ 50, -2 ≤ logD ≤ 5.

Table S2. Geometry optimization and conformational stability of **7**.

No.	Conformer	ΔE (eV)	ΔE (kcal/mol)
1	EE	Minimum	Minimum
2	ZE	0.05	1.18
3	ZZ	0.18	4.15
4	EZ	0.21	4.90
5	Enol-thiazole	0.75	17.23