Supporting Information

Synthesis, Characterization, Biological Activities and Computational Studies of Pyrazolyl-Thiazole Derivatives of Thiophene

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1. Biological Activity

Disk diffusion assay

The antituberculosis potential of synthesized compounds was evaluated using **Kirby-Bauer disk diffusion** method.[1,3] Briefly, each sterile disk (Himedia Pvt. Ltd. Mumbai) loaded with synthesized compounds 50 µL (1 mg/ mL) and dried. Each disk was then placed on the surface of the sterile solidified Middlebrook 7H9 agar medium which was spreaded with 24 hrs old inoculums *M. Tuberculosis H₃₇Ra* and Rifampicin (1mg/ mL). The plates were kept in refrigerator for diffusion for 1 hr and then transferred to the incubator at 37ºC for 24-48 hrs. After incubation, the zones around the discs were measured by the zone scale (Himedia Pvt. Ltd. Mumbai).

Resazurin microtiter assay (REMA) for calculating MIC

The REMA plate assay was carried out as described elsewhere.[2,3] Briefly, 100 µL of Middlebrook 7H9 broth medium was dispensed in each well of a sterile flat-bottom 96-well plate, and serial twofold dilutions of each synthesized compounds were prepared directly in the plate. Fifty micro litres of inoculums was added to each well. Sterile cold water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed with the sterile plastic bag, and incubated at 37°C for 24 hrs. After 24 hrs of incubation, 30 µL of resazurin solution (0.01% in sterile deionized water) was added to each well, and the plate was re-incubated for 12 hrs. A change in color from blue to pink indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color. The drug concentration ranges used were as follows: for synthesized compounds and standards 0.97- 500 µg/mL.

DPPH (2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay)

Free radical damage is one of the important aspect in the tuberculosis management. DPPH is stable reagent used in this spectrophotometric assay.[4] Briefly, the assay was performed by mixing of equal quantity of DPPH solution and the test compound, so that the final volume is made up to 3 mL incubate the samples for 20 min, read the absorbance at 517 nm using (Shimadzu UV Vis- Spectrophotometer). Ascorbic acid (1 mM) was used as a standard. Percent inhibition was calculated using following formula:

% radical scavenging activity = 1 – T/C x 100

OH (Hydroxyl radical assay)

The OH radical scavenging activity was demonstrated with Fenton reaction.[3,4] Briefly, the typical reaction mixture contained 60 µL of $FeCl_2$ (1 mM), 90 µL of 1-10 phenanthroline (1 mM), and 2.4 mL of phosphate buffer (0.2 M, pH 7.8), 150 µL of H_2O_2 (0.17 M) and 1.5 mL Of individual synthesized compound (1 mg/mL). The reaction was started by adding H_2O_2. After 5 min incubation at room temperature, the absorbance was read at 560 nm. Ascorbic acid (1 mM) was used as reference.

% radical scavenging activity = 1 – T/C x 100

2. Computational Methodology

All electronic structure calculations were performed using the Gaussian 16 software package.[4] Geometry optimizations for all compounds were performed in the gas phase using the B3LYP functional with the 6-31G(d) basis set. Harmonic frequency calculations confirmed the absence of imaginary frequencies, ensuring that all optimized structures correspond to local minima on the potential energy surface.

Subsequent single-point energy calculations were carried out using the ωB97X-D[5] functional with the Ahlrichs def2-TZVPP[6] basis set for all atoms (H, C, N, O, S, F, Cl, and Br). Thermal corrections for Gibbs free energy were calculated from thermochemistry analyses considering an ideal gas at 298.15 K and 1 atm.

Gas-phase calculations for absorption and emission spectra of all compounds were performed using the τ-HCTHhyb[7] density functional for the ground and first excited (singlet) states, respectively. Key computational results, including optimized geometries and energy values, are provided in Table S1 and Figure S1.

Geometry optimizations were carried out in the gas phase to focus on the intrinsic molecular properties and reduce computational demands. These results are expected to be reliable for the compounds studied, based on comparisons with similar systems in the literature.[8]
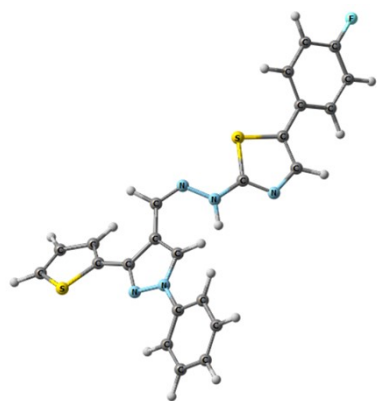
4
(0.0)
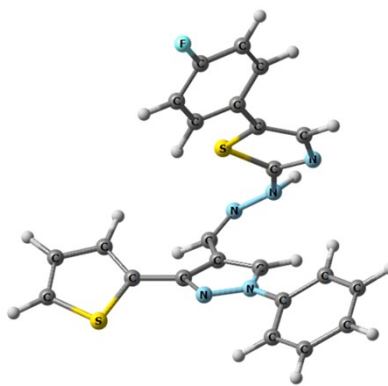
4cis
(5.3)
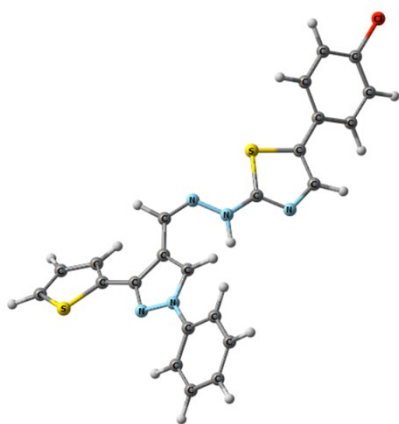
5
(0.0)

5cis
(5.1)

6
(0.0)

6cis
(5.5)

Figure S1. Conformational study of the species **1-6**. Energies shown are in Gibbs free energies (kcal/mol).

Table S1. Cartesian coordinates (x-y-z format) of the calculated geometry optimizations performed at the ωB97X-D/def2tzvpp level.

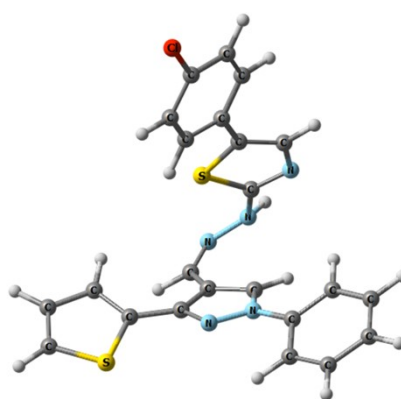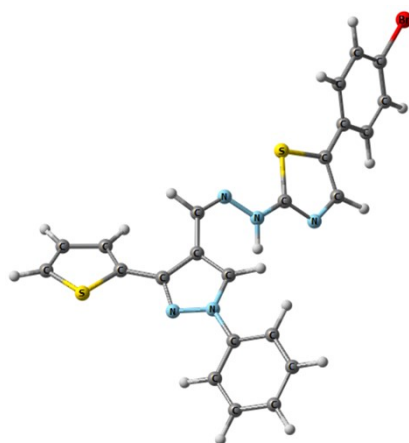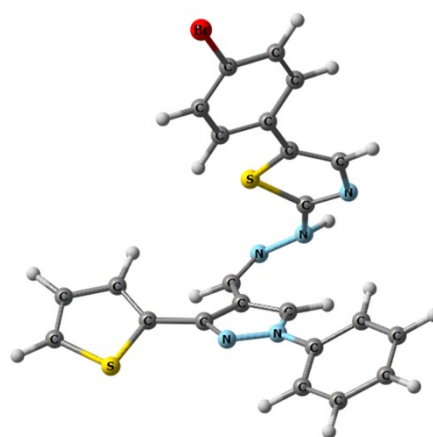| 1				1cis			
E(scf) = -2161.33133142 a.u.				E(scf) = -2161.32289107 a.u.			
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C	2.763712	0.545601	-0.516755	C	2.529996	0.306252	1.114242
C	2.996078	-0.820073	-0.586164	C	2.304371	-1.054487	0.970118
N	4.293525	-1.023085	-0.253696	N	2.372884	-1.329479	-0.349309
H	2.340661	-1.641214	-0.868648	H	2.133721	-1.826107	1.716477
N	4.919578	0.118902	0.047071	N	2.621232	-0.238779	-1.086082
C	4.985467	-2.261679	-0.166207	C	2.189594	-2.591698	-0.975550
C	4.556496	-3.364141	-0.907753	C	1.397582	-3.566592	-0.365782
C	6.098068	-2.359444	0.671811	C	2.803191	-2.837796	-2.204859
C	5.234117	-4.576296	-0.789941	C	1.241223	-4.805893	-0.983757
H	3.710514	-3.281433	-1.594194	H	0.883768	-3.357421	0.575939
C	6.772951	-3.573013	0.771634	C	2.628894	-4.075979	-2.817128
H	6.421006	-1.480310	1.231281	H	3.403165	-2.052602	-2.667537
C	6.342383	-4.686100	0.048682	C	1.854881	-5.065086	-2.208702
H	4.896188	-5.438206	-1.371659	H	0.619904	-5.568541	-0.506542
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H	6.872894	-5.637976	0.135377	H	1.723730	-6.036195	-2.693411
C	4.377605	2.477610	0.133351	C	2.958110	2.127533	-0.688488
C	3.556840	3.532419	0.460627	C	2.546538	3.311026	-0.119749
S	6.031239	2.982407	0.039039	S	3.836108	2.414322	-2.152349
C	4.269583	4.754041	0.629205	C	2.950832	4.453091	-0.868655
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C	5.615480	4.601885	0.430030	C	3.657258	4.114849	-1.991484
H	3.800195	5.703341	0.893576	H	2.718820	5.482253	-0.588393
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C	1.564472	1.300034	-0.889433	C	2.654971	1.091024	2.336557
H	1.714732	2.262301	-1.392184	H	3.289319	1.984434	2.275395
N	0.338831	0.975643	-0.707114	N	2.178149	0.877640	3.508276
N	0.039749	-0.164278	-0.060427	N	1.331393	-0.198614	3.685480
H	0.733553	-0.660262	0.500618	H	1.303220	-0.493713	4.657117
C	-1.273690	-0.449522	0.205575	C	0.107031	-0.353654	3.073162
S	-2.549749	0.521390	-0.464141	S	-0.503855	0.711051	1.833643
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C	-3.698057	-0.537142	0.331741	C	-1.979342	-0.221741	1.805467
H	-3.471400	-2.306455	1.587792	H	-2.642980	-1.985909	2.899966
N	-1.642884	-1.451259	0.945337	N	-0.708978	-1.306013	3.429703
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C	-5.706107	0.367353	-0.855180	C	-2.821561	0.727242	-0.350612
C	-6.015015	-0.891422	1.182553	C	-4.382573	-0.332586	1.160019
C	-7.080081	0.535832	-0.968690	C	-3.850806	0.979687	-1.248445
H	-5.055222	0.791618	-1.625001	H	-1.804289	1.035423	-0.609204

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C	-7.908104	-0.024864	-0.002079	C	-5.138345	0.560520	-0.929867
H	-7.519014	1.088376	-1.800215	H	-3.667905	1.487968	-2.195885
H	-8.069621	-1.155393	1.819977	H	-6.441893	-0.399487	0.485200
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N	4.165724	1.097061	0.213768	N	-0.296915	0.251769	-1.655554
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N	4.814431	-0.002425	-0.182219	N	-0.977739	-0.827380	-1.254968
C	4.790487	2.369913	0.114455	C	1.102152	0.166329	-1.874490
C	4.389677	3.419459	0.943290	C	1.877924	1.324882	-1.979742
C	5.808057	2.554956	-0.823622	C	1.707233	-1.091051	-1.939092
C	4.998828	4.666428	0.814335	C	3.257633	1.218565	-2.145190
H	3.619985	3.268103	1.703874	H	1.420851	2.313868	-1.912847
C	6.415808	3.802560	-0.934751	C	3.087164	-1.182098	-2.102348
H	6.111364	1.715937	-1.451651	H	1.087694	-1.983001	-1.842748
C	6.011623	4.863322	-0.123240	C	3.869858	-0.032065	-2.206111
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H	7.211248	3.946953	-1.671036	H	3.558362	-2.168037	-2.140978
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S	6.044925	-2.821379	-0.344996	S	-2.750127	-2.996282	-0.171893
C	4.317073	-4.638616	-0.894691	C	-5.140020	-2.274838	0.427469
H	2.483605	-3.391401	-0.704431	H	-4.985391	-0.141450	-0.187056
C	5.666329	-4.440251	-0.775225	C	-4.311661	-3.363279	0.447599
H	3.867570	-5.594847	-1.168519	H	-6.173785	-2.294972	0.777739
H	6.463891	-5.169099	-0.923407	H	-4.540375	-4.371802	0.793652
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H	1.842115	-2.348863	1.426752	H	-4.338080	1.745201	-1.797732
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H	0.567463	0.598320	-0.243402	H	-3.229735	3.954147	1.264461
C	-1.396969	0.254463	0.180507	C	-1.684768	2.610137	1.093732
S	-2.563378	-0.831601	0.874673	S	-1.383765	1.036944	1.766041
C	-3.241093	1.227798	-0.461707	C	0.500091	2.530182	0.986594
C	-3.824594	0.188559	0.211846	C	0.303315	1.248527	1.449586
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C	-5.708588	-0.892636	1.467494
C	-6.198569	0.415195	-0.494295
C	-7.065435	-1.155943	1.642794
H	-4.989104	-1.300962	2.183696
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H	-5.903850	1.011956	-1.358056
C	-8.007315	-0.631889	0.762099
H	-7.392926	-1.774197	2.482587
H	-9.075689	-0.817178	0.875099
O	-8.098315	1.389286	-2.152861
N	-8.524197	0.709213	-1.245661
O	-9.693180	0.454105	-1.059293

N	-0.616868	3.280472	0.781547
C	1.269455	0.143288	1.513299
C	2.628756	0.386944	1.282334
C	0.849065	-1.184819	1.683216
C	3.507953	-0.680857	1.201887
H	3.017923	1.393123	1.130718
C	1.757681	-2.239724	1.632900
H	-0.212102	-1.407435	1.826057
C	3.103803	-2.000224	1.379293
H	1.401606	-3.264008	1.766979
H	3.832379	-2.807405	1.301549
N	4.913644	-0.404329	0.861241
O	5.631889	-1.354849	0.640754
O	5.262619	0.754451	0.802148

3

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C	-3.831755	-1.036138	0.074281
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C	-2.726536	0.833818	0.502958
N	-4.015832	1.080181	0.165611
H	-2.054444	1.629670	0.817383
N	-4.690636	-0.043461	-0.095985
C	-4.662879	2.341479	0.085471
C	-3.920273	3.499047	-0.156415
C	-6.047746	2.408967	0.252238
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C	-6.683800	3.645462	0.186336
H	-6.606041	1.487900	0.426521
C	-5.949171	4.810755	-0.037848
H	-3.984487	5.636713	-0.399474
H	-7.768085	3.698206	0.317773
H	-6.454057	5.779162	-0.085968
C	-4.237624	-2.422239	-0.136668
C	-3.458635	-3.511914	-0.451013
S	-5.907702	-2.866142	-0.020038
C	-4.215894	-4.710205	-0.590649
H	-2.379965	-3.452351	-0.608162
C	-5.553628	-4.506935	-0.383399
H	-3.782375	-5.680199	-0.840893
H	-6.356180	-5.243708	-0.430154
C	-1.376866	-1.328283	0.860355
H	-1.562777	-2.282865	1.365210
N	-0.139310	-1.035176	0.701549
N	0.203063	0.093522	0.063225

3cis

E(scf) = -2071.33421540 a.u.

C	2.531472	0.732135	-0.219701
C	2.351729	0.324376	1.138307
C	2.109881	-1.037028	1.043093
N	2.160921	-1.358594	-0.267693
H	1.934797	-1.780112	1.816576
N	2.414836	-0.297493	-1.043850
C	1.949127	-2.638020	-0.844931
C	1.147598	-3.575741	-0.190156
C	2.544871	-2.940121	-2.070502
C	0.965249	-4.834208	-0.760872
H	0.647718	-3.320932	0.748214
C	2.344577	-4.197088	-2.634464
H	3.152218	-2.182264	-2.567959
C	1.561515	-5.149918	-1.981200
H	0.336907	-5.568011	-0.248714
H	2.811004	-4.434431	-3.594601
H	1.410098	-6.136356	-2.427813
C	2.777909	2.076181	-0.734267
C	2.403784	3.285522	-0.195563
S	3.611627	2.296644	-2.235484
C	2.801375	4.393870	-0.997616
H	1.830688	3.377937	0.729126
C	3.465055	4.004907	-2.129946
H	2.594656	5.435999	-0.746758
H	3.873480	4.640523	-2.916337
C	2.485789	1.144350	2.338786
H	3.139074	2.022226	2.259764
N	1.989338	0.969182	3.509562
N	1.127737	-0.086417	3.703929

H	-0.464374	0.596592	-0.522818	H	1.010174	-0.288204	4.691845
C	1.531735	0.345351	-0.191179	C	-0.035788	-0.282283	2.971213
S	2.783815	-0.631288	0.517193	S	-0.726581	0.940887	1.928856
C	3.296033	1.347187	-0.997714	C	-1.853857	-1.301506	2.281259
C	3.961669	0.387710	-0.286067	C	-2.035644	-0.149422	1.566464
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C	5.408476	0.170613	-0.149927	C	-3.092990	0.189620	0.602740
C	5.951415	-1.112228	0.045554	C	-2.812394	0.909405	-0.572842
C	6.300030	1.246550	-0.222983	C	-4.411943	-0.218924	0.821720
C	7.318997	-1.305958	0.158424	C	-3.810745	1.201242	-1.487795
H	5.286704	-1.980363	0.097432	H	-1.787000	1.231107	-0.780470
C	7.679663	1.064267	-0.125116	C	-5.424721	0.059991	-0.097069
H	5.910483	2.261390	-0.343436	H	-4.662860	-0.756482	1.740727
C	8.201050	-0.219345	0.069725	C	-5.128926	0.777072	-1.261427
H	8.331833	1.937367	-0.186853	H	-6.440618	-0.276856	0.117045
H	7.738048	-2.303787	0.308575	H	-3.592931	1.754915	-2.404111
O	9.514698	-0.504635	0.186844	O	-6.031759	1.105257	-2.207783
C	10.450088	0.532614	0.091653	C	-7.367889	0.721635	-2.039618
H	10.312161	1.284423	0.892335	H	-7.810965	1.171741	-1.130742
H	11.441016	0.069280	0.205018	H	-7.910667	1.090601	-2.921936
H	10.399869	1.038984	-0.891332	H	-7.474754	-0.378730	-1.986392

	4				**4cis**		
	E(scf) = -2056.06229482 a.u.				E(scf) = -2056.05419211 a.u.		
C	-3.437881	-1.065927	-0.099593	C	2.359893	0.355373	-0.121841
C	-2.159057	-0.548409	-0.480316	C	2.063919	0.051205	1.242805
C	-2.365587	0.821584	-0.534848	C	1.574616	-1.243993	1.191567
N	-3.665438	1.045970	-0.222586	N	1.599515	-1.627251	-0.103047
H	-1.688580	1.630237	-0.800056	H	1.239965	-1.907639	1.984337
N	-4.317090	-0.089042	0.050126	N	2.072938	-0.669579	-0.910204
C	-4.338370	2.294789	-0.132612	C	1.128835	-2.855985	-0.635880
C	-3.808589	3.431539	-0.747400	C	0.118563	-3.555964	0.027491
C	-5.537176	2.371103	0.580168	C	1.673997	-3.343722	-1.824472
C	-4.473358	4.650501	-0.629345	C	-0.329679	-4.766928	-0.496219
H	-2.888078	3.376481	-1.332654	H	-0.335450	-3.148007	0.934685
C	-6.196436	3.593183	0.681274	C	1.208360	-4.549528	-2.342419
H	-5.938513	1.468977	1.043326	H	2.450513	-2.767487	-2.330445
C	-5.667693	4.737768	0.084095	C	0.211621	-5.267524	-1.679813
H	-4.053056	5.537665	-1.110674	H	-1.122480	-5.313990	0.021354
H	-7.135236	3.649646	1.239141	H	1.634145	-4.934045	-3.273401
H	-6.186991	5.695703	0.171692	H	-0.149057	-6.214128	-2.091318
C	-3.821270	-2.457545	0.117615	C	2.874316	1.605960	-0.673140
C	-3.028094	-3.530695	0.453113	C	2.704123	2.886721	-0.200305
S	-5.481639	-2.931034	-0.018761	S	3.810414	1.600714	-2.128797
C	-3.766714	-4.740480	0.593296	C	3.340057	3.865592	-1.017216

H	-1.953029	-3.451450	0.625386	H	2.112896	3.124092	0.686537
C	-5.104664	-4.561783	0.365363	C	3.976970	3.309923	-2.094119
H	-3.320518	-5.700669	0.858568	H	3.316548	4.938302	-0.816001
H	-5.895136	-5.311783	0.407070	H	4.535697	3.822763	-2.877758
C	-0.968961	-1.319987	-0.846925	C	2.284351	0.896786	2.412861
H	-1.132050	-2.285749	-1.338396	H	3.101080	1.626022	2.345120
N	0.261378	-1.004723	-0.678112	N	1.682979	0.894441	3.547446
N	0.580107	0.140341	-0.054653	N	0.619725	0.041700	3.728411
H	-0.098465	0.643972	0.517850	H	0.393612	-0.068511	4.711702
C	1.903258	0.413634	0.199625	C	-0.488673	-0.017376	2.896992
S	3.165485	-0.579443	-0.467211	S	-0.888075	1.238949	1.745474
C	3.655719	1.460340	0.973138	C	-2.365857	-0.795503	2.065180
C	4.331047	0.482895	0.295480	C	-2.300154	0.329459	1.289578
H	4.139292	2.270568	1.523980	H	-3.145045	-1.555799	1.972775
N	2.288349	1.419856	0.919726	N	-1.343211	-0.993501	2.951242
C	5.780552	0.275327	0.170926	C	-3.190560	0.755456	0.199057
C	6.340111	-0.327825	-0.966413	C	-2.683979	1.359171	-0.962351
C	6.648558	0.692515	1.193244	C	-4.573632	0.539630	0.292770
C	7.715313	-0.506863	-1.087055	C	-3.529832	1.736304	-2.001132
H	5.689451	-0.654605	-1.783209	H	-1.606869	1.521999	-1.065858
C	8.026100	0.532507	1.079202	C	-5.428466	0.902146	-0.744310
H	6.237506	1.136253	2.103870	H	-4.989976	0.094079	1.200311
C	8.544311	-0.068227	-0.062125	C	-4.894113	1.499370	-1.879829
H	8.704685	0.855071	1.872281	H	-6.506658	0.739719	-0.677200
H	8.152954	-0.974939	-1.971765	H	-3.141603	2.202544	-2.909478
F	9.862928	-0.231580	-0.172424	F	-5.708489	1.856150	-2.872296

5				**5cis**			
E(scf) = -2416.41832623 a.u.				E(scf) = -2416.41007845 a.u.			
C	-3.792866	-1.073043	-0.103379	C	-2.495822	-0.732796	-0.212962
C	-2.515428	-0.550403	-0.481469	C	-2.339368	-0.288368	1.136276
C	-2.727645	0.818680	-0.538258	C	-2.093273	1.069956	1.008512
N	-4.029135	1.037714	-0.229651	N	-2.120907	1.355582	-0.310987
H	-2.053447	1.630035	-0.802487	H	-1.933255	1.835320	1.763293
N	-4.676609	-0.099721	0.042963	N	-2.362348	0.274087	-1.062313
C	-4.707785	2.283786	-0.143465	C	-1.902147	2.619839	-0.919530
C	-4.184284	3.420361	-0.763838	C	-1.113979	3.577428	-0.277669
C	-5.905632	2.357233	0.571144	C	-2.478231	2.886252	-2.162528
C	-4.854395	4.636746	-0.649368	C	-0.925400	4.820577	-0.879011
H	-3.264734	3.366824	-1.350789	H	-0.628990	3.350428	0.675424
C	-6.570345	3.576656	0.668553	C	-2.271856	4.128074	-2.757026
H	-6.302036	1.455034	1.038422	H	-3.075227	2.113136	-2.648946
C	-6.047827	4.721253	0.065923	C	-1.502391	5.100781	-2.117178
H	-4.439119	5.523942	-1.134968	H	-0.307713	5.570177	-0.376924
H	-7.508438	3.631065	1.227803	H	-2.723090	4.337798	-3.730730
H	-6.571348	5.677146	0.150631	H	-1.346559	6.075356	-2.587560

C	-4.170545	-2.466032	0.114660	C	-2.735921	-2.089873	-0.695042
C	-3.373046	-3.535545	0.451565	C	-2.372584	-3.284969	-0.118334
S	-5.828792	-2.946502	-0.022416	S	-3.546068	-2.349032	-2.202831
C	-4.106720	-4.748261	0.592364	C	-2.758907	-4.413652	-0.897018
H	-2.298445	-3.451693	0.624601	H	-1.815396	-3.353983	0.818160
C	-5.445257	-4.575286	0.363383	C	-3.403629	-4.054081	-2.049830
H	-3.656755	-5.706358	0.858813	H	-2.558003	-5.448944	-0.615197
H	-6.232679	-5.328469	0.405293	H	-3.800626	-4.709958	-2.825368
C	-1.321168	-1.316982	-0.844981	C	-2.493407	-1.078744	2.353618
H	-1.478756	-2.282264	-1.339266	H	-3.150061	-1.955185	2.286119
N	-0.092793	-0.997750	-0.669924	N	-2.010350	-0.881076	3.526378
N	0.217750	0.146803	-0.040497	N	-1.143379	0.173280	3.706735
H	-0.466180	0.645630	0.529883	H	-1.059098	0.423953	4.686842
C	1.537727	0.426784	0.218170	C	0.036149	0.346951	3.003356
S	2.807315	-0.554371	-0.451405	S	0.707977	-0.868291	1.940886
C	3.280790	1.479293	1.003972	C	1.902020	1.323784	2.386179
C	3.964345	0.509561	0.322351	C	2.062560	0.185856	1.643191
H	3.757209	2.289066	1.561591	H	2.597395	2.166317	2.370467
N	1.914793	1.431671	0.945445	N	0.762335	1.416535	3.134633
C	5.414364	0.313139	0.199664	C	3.123335	-0.151980	0.684019
C	5.977393	-0.317934	-0.920221	C	2.851047	-0.899123	-0.472072
C	6.282410	0.767327	1.205732	C	4.437211	0.292275	0.895559
C	7.353581	-0.487359	-1.039496	C	3.853723	-1.189116	-1.392134
H	5.329241	-0.675102	-1.726285	H	1.832249	-1.246667	-0.669059
C	7.660356	0.615293	1.092065	C	5.445355	0.017850	-0.023578
H	5.872624	1.233633	2.105560	H	4.679991	0.849788	1.804342
C	8.192188	-0.014088	-0.032551	C	5.148086	-0.724147	-1.165723
H	8.325571	0.971942	1.881512	H	6.465464	0.367669	0.149253
H	7.778320	-0.978580	-1.917602	H	3.630646	-1.766074	-2.292167
Cl	9.916075	-0.215646	-0.175590	Cl	6.405226	-1.075145	-2.317906

6				**6₍cis₎**			
E(scf) = -4530.42175374 a.u.				E(scf) = -4530.41369373 a.u.			
C	-4.504111	-1.081474	-0.107426	C	-2.852730	-0.952321	-0.332673
C	-3.226618	-0.552819	-0.476704	C	-2.920255	-0.361820	0.967127
C	-3.444896	0.815257	-0.535322	C	-2.802502	0.996390	0.717235
N	-4.749503	1.028083	-0.235950	N	-2.687022	1.145274	-0.619523
H	-2.772684	1.629841	-0.794701	H	-2.819869	1.844987	1.395836
N	-5.393476	-0.112318	0.032554	N	-2.715259	-0.025663	-1.268735
C	-5.434880	2.270891	-0.156031	C	-2.503247	2.362181	-1.327542
C	-4.915360	3.407875	-0.778957	C	-1.881289	3.442777	-0.698326
C	-6.635209	2.340577	0.554721	C	-2.943075	2.461066	-2.648447
C	-5.592042	4.621193	-0.670772	C	-1.724611	4.639807	-1.394042
H	-3.993950	3.356665	-1.363216	H	-1.498279	3.345906	0.321184
C	-7.306507	3.556882	0.645807	C	-2.769167	3.659593	-3.335565
H	-7.028404	1.437954	1.023924	H	-3.410053	1.595185	-3.120766

C	-6.788022	4.702012	0.040690	C	-2.167574	4.753357	-2.711432
H	-5.179987	5.508798	-1.158362	H	-1.237688	5.486401	-0.902374
H	-8.246527	3.608481	1.202071	H	-3.113565	3.740173	-4.370338
H	-7.316725	5.655485	0.120362	H	-2.037424	5.693337	-3.254361
C	-4.876679	-2.476205	0.108077	C	-2.884411	-2.370849	-0.677469
C	-4.076259	-3.542337	0.448750	C	-2.451088	-3.443496	0.067505
S	-6.531993	-2.963956	-0.038405	S	-3.494705	-2.887600	-2.212259
C	-4.805308	-4.758377	0.584884	C	-2.620821	-4.687769	-0.605120
H	-3.002965	-3.453860	0.627506	H	-1.991143	-3.340774	1.052551
C	-6.143338	-4.591196	0.348696	C	-3.175444	-4.535264	-1.847472
H	-4.352598	-5.714625	0.853321	H	-2.334901	-5.653558	-0.184296
H	-6.927673	-5.347823	0.385992	H	-3.411222	-5.313022	-2.574505
C	-2.026492	-1.313440	-0.833310	C	-3.143628	-1.037863	2.241379
H	-2.176464	-2.278669	-1.330110	H	-3.677140	-1.995657	2.200054
N	-0.800831	-0.988636	-0.649919	N	-2.842971	-0.659645	3.430937
N	-0.500282	0.156086	-0.015540	N	-2.134715	0.514334	3.577157
H	-1.190159	0.649038	0.552799	H	-2.200870	0.873623	4.524408
C	0.816574	0.443705	0.249248	C	-0.900025	0.744672	2.992826
S	2.094891	-0.523689	-0.423362	S	0.049024	-0.518314	2.242597
C	2.549618	1.503654	1.046999	C	0.896347	1.865258	2.414170
C	3.242126	0.543390	0.360720	C	1.289389	0.656882	1.906303
H	3.018564	2.313000	1.611520	H	1.477399	2.785900	2.322732
N	1.184420	1.446694	0.984145	N	-0.334452	1.914111	3.007144
C	4.693498	0.358028	0.239419	C	2.501060	0.317618	1.147203
C	5.261417	-0.267629	-0.880879	C	2.479374	-0.655854	0.137224
C	5.557839	0.816529	1.246576	C	3.710752	0.977881	1.408987
C	6.638994	-0.427978	-0.999290	C	3.625291	-0.962388	-0.590333
H	4.616617	-0.627835	-1.688292	H	1.545721	-1.175433	-0.099005
C	6.937177	0.673179	1.133748	C	4.860389	0.686794	0.680298
H	5.144900	1.279206	2.146859	H	3.760188	1.721509	2.209007
C	7.474706	0.048594	0.008797	C	4.813408	-0.286460	-0.316967
H	7.593995	1.035525	1.928039	H	5.795906	1.207403	0.897521
H	7.061156	-0.916020	-1.880646	H	3.590184	-1.721153	-1.375171
Br	9.348197	-0.159545	-0.146471	Br	6.373649	-0.698292	-1.302456

2$_{iso}$

E(scf) = -2161.32995310 a.u.

C	-3.809182	1.127668	0.094900
C	-2.547367	0.555124	0.451463
C	-2.829269	-0.795461	0.590405
N	-4.151940	-0.957222	0.343776
H	-2.189644	-1.629653	0.870070
N	-4.748549	0.198328	0.034274
C	-4.899503	-2.166344	0.349755
C	-4.439510	-3.280265	1.055258
C	-6.100437	-2.224567	-0.360660

C	-5.177379	-4.462265	1.029434
H	-3.518887	-3.232231	1.641284
C	-6.833305	-3.408206	-0.368907
H	-6.445363	-1.337811	-0.894031
C	-6.374672	-4.532118	0.318937
H	-4.813469	-5.332673	1.582044
H	-7.773902	-3.451512	-0.924865
H	-6.951841	-5.460470	0.305429
C	-4.118476	2.526409	-0.184598
C	-3.275204	3.529126	-0.604989
S	-5.741136	3.107790	-0.018017
C	-3.944944	4.772902	-0.786967
H	-2.213596	3.375332	-0.807733
C	-5.281894	4.689233	-0.504416
H	-3.453234	5.688289	-1.121178
H	-6.028050	5.482394	-0.561356
C	-1.298384	1.271591	0.722379
H	-1.380255	2.259333	1.190204
N	-0.099699	0.886556	0.486864
N	0.116328	-0.292359	-0.120795
H	-0.627411	-0.781301	-0.620658
C	1.400269	-0.638275	-0.456966
S	2.750806	0.312655	0.084208
C	3.037905	-1.786105	-1.329355
C	3.805516	-0.821102	-0.734773
H	3.439880	-2.632517	-1.891341
N	1.684764	-1.682706	-1.173143
C	5.266209	-0.679668	-0.729799
C	6.040427	-1.219696	-1.771833
C	5.928061	-0.013088	0.310152
C	7.428771	-1.114502	-1.768350
H	5.542994	-1.714981	-2.609733
C	7.313100	0.090105	0.284919
H	5.382475	0.420740	1.150133
C	8.086670	-0.452225	-0.734511
H	8.006347	-1.543332	-2.591303
H	9.171369	-0.345381	-0.709414
O	7.292123	1.265973	2.264912
N	7.988077	0.797543	1.391698
O	9.196701	0.865033	1.355814

3. Molecular Docking

Molecular docking studies were conducted to understand the binding interactions of the synthesized compounds with potential biological targets. The docking results revealed that the compounds fit well into the active sites of the target proteins, forming stable interactions that support their observed antimicrobial. The binding affinities and interaction details provided insights into the mechanisms of action of the compounds, suggesting that they could serve as leads for further drug development.

Molecular docking studies of compounds **7a-g** and penicillin as the reference drug were performed with penicillin-binding proteins from gram-positive (*S. aureus y B. subtillis*) and gram-negative (*E. coli*) bacteria were performed. Fluconazole as a reference drug on the fungal protein sterol 14α-demethylase of *C. albicans*; all pathogenic microorganisms of medical interest. To understand the affinity with which ligands bind at the active site of the proteins, the affinity energy was determined as show in Figure 3. A more negative value indicates a higher ligand-receptor binding affinity; however, to have a pharmacological affect in addition to affinity, interaction with specific amino acids is also required to inhibit or activate pharmacological targets, as explained in Figure 4. Where the binding modes of compounds **7d** and **7g** in the active sites of bacterial proteins are described, these two molecules being the ones that presented the best inhibitory activity according to the results shown in Table 1 and Figure 1 (Manuscript). Likewise, the interactions of **7g** on sterol 14α-demethylase, a compound with the best fungal inhibition activity, are described.

Methicillin-resistant *S. aureus* (MRSA) infection is a leading cause of mortality and morbidity among bacterial diseases. Penicillin-binding protein (PBP) play a primary role in building the bacterial cell wall. In this sense, MRSA expresses PBP2a (Figure 4A1) resistant to the action of methicillin and other b-lactams; protein on which molecular docking were carried out. Figure 3 (Manuscript) show the affinity energies ($\Delta G = kcal/mol$) of the binding of compounds **7a-g** and penicillin at the active site of PBP2a, observing that penicillin has an affinity energy of -8.18 *kcal*/mol and the compounds under study have similar energies (-8.14, -7.89, -7.92, -8.21, -8.08, -8.10 and -8.15 respectively **7a-g**), it should be noted that compounds **7d** and g are the ones with the

best affinity energies (Table S2), which is consistent with the inhibitory activity on this microorganism reported in Table 2 (Manuscript).

Table S2. Affinity energy (ΔG, *kcal/mol*) of the molecules under study at the active site of bacterial penicillin-binding protein and fungal Lanosterol 14α-demethylase

Molecule	S. aureus 1MWT	B. subtillis 1W5D	E. coli 2EX8	C. albicans 5TZ1
7a	-8.14	-8.76	-8.07	-10.38
7b	-7.89	-8.22	-7.56	-10.35
7c	-7.92	-7.95	-7.25	-10.33
7d	-8.21	-8.09	-7.83	-10.40
7e	-8.08	-8.19	-7.91	-9.94
7f	-8.10	-8.16	-7.86	-10.25
7g	-8.15	-8.30	-8.05	-10.49
Penicillin	-8.18	-8.90	-8.24	-----
Fluconazole	-----	-----	-----	-8.33

According to Lim and Strynadka,[9] the amino acids that form the active site of PBP2a are Ser403, Lys406, Tyr446, Ser462, Asn464, Ser598, Gly599, Thr600 y Met641; Ser403 being the catalytic base with which inhibitors interact. Figure 4A2 show that penicillin interacts at the active site with the amino acids Ser403, Lys406, Ser462, Ser598 with hydrogen bonding interaction, while Tyr446 and Met641 with a π-sulfur interaction. In the case of compounds **7d** (Figure 4A3) it interacts through a hydrogen bond with the Ser462 residue, with interaction of a π-donor hydrogen bond with the amino acids Tyr446 and Asn464, while Thr600 with a C-H bond and π-π stacking, also with Met641 whit π-π interaction. The molecule **7g** (Figure 4A4) interacts with H-bond with the amino acids Ser403 and Tyr446, Asn464 interacts with the ligand with a π-donor hydrogen bond and Thr600 with a C-H bond and the Met641 residue with π-π stacks.

To understand the binding mode of penicillin and **7a-g** compounds in the active site of the penicillin-binding protein (PBP) of *E. coli*, PBP4 (Figure 4B1) was used, which has both endopeptidase and carboxypeptidase activity, which is one of the 12 PBPs that participate in the synthesis and maintenance of the cell wall of the pathogen. We evaluated the affinity of penicillin on the active site of PBP4 by finding a binding energy of -8.24 *kcal*/mol while the values of the binding affinities of the **7a-g** compounds are similar (-8.07, -7.56, -7.25, -7.83, -7.91, -7.86, -8.05 *kcal/mol*, respectively), as shown in Figure 3.

According to Kishida, H., et al.,[10] the active site is made up of the residues Ser62, Asn154, Asp155, Met156, Thr157, Gln158, Cys159, Phe160, Ser161, Lys305, Ser306, Asp307, Asn308, Gly419, Ser420, Leu421. In this sense, Figure 4B2 shows the mode of binding of penicillin at the active site of PBP4, it interacts with hydrogen bonds, the main residue Ser62, and with the amino acids Lys65, Ser306, Asn308, Ser398 and Lys417. It also interacts with Gly419 and Ser420 with carbon hydrogen bonds. Similarly, **7d** (Figure 4B3) interacts with Ser62 by hydrogen bonds, with π-π stacking with Phe160, π-cation interaction with Arg361, with Leu359 and Leu421 through π-alkyl interactions, with carbon hydrogen bonds with the Gly419 and Thr418 residue, while Gln422 interacts with a π-donor hydrogen bond and Ser398 presents a halogen bridge. Figure 4B4 shows the interactions of **7g**, it forms a H-bond with Ser62, π-π stacking with Phe160, while with Leu421 it is through π-alkyl interactions, π-cation interaction with Arg361, with a π-donor H-bond, it bonds with Thr418 and Gly419 with C-H bonds.

In Figure 3, we can see that penicillin has a discrete but higher affinity energy (-8.90 *kcal*/mol) compared to the **7a-g** compounds under study (-8.76, -8.22, -7.95, -8.09, -8.19, -8.16 y -8.30 *kcal*/mol, respectively), when interacting at the active site of the penicillin-binding protein of *B. subtillis* PBP4a (Figure 4C1), which, it may be related to their increased biological activity.

The active site PBP4a is formed by the residues Ser52, Lys55, Asp145, Tyr150, Ser299, Asn301, His352, Thr394, Arg398, Lys411, Thr412 and Ser414.[11] It should be noted that the central amino acid is Ser52 and that it is important in the inhibition of this protein. Figure 4C2 show the interactions of penicillin at the active site of PBP4a. The residues Ser52, Lys55, Asn301, Lys411, Thr412, Lys449 by hydrogen bonds,

while by carbon hydrogen bond interacts with Thr394, Ser414 and Ser299, with the latter amino acid also presents a π-lone pair interaction, with Tyr149 it forms a π-donor hydrogen bond.

The binding mode and interactions of ligand **7d** at the active site of PBP4a are shown in Figure 4C3. It interacts with Ser52 forming a H-bond and a carbon hydrogen bond, with Thr149 it forms a π-donor H-bond and a π-π stacking with Tyr150, with Ser414 a sulphur-x interaction is formed that occurs between the divalent sulphur and O, N or S atoms, while it joins with a halogen bridge with Thr394. The **7g** compound's bond in similar manner as seen in Figure 4C4. It interacts with Ser52 by forming H-bonds and a C-H bond, with Tyr149 through a π-donor H-bond, and with a π-π stacking with Tyr150, in the same way that **7d** forms a sulphur-x interaction with Ser414.

To understand the antifungal biological activity of synthesized compounds **7a-g** and fluconazole as a reference, the binding mode at the active site of the protein sterol 14α-demethylase or CYP51 of *C. albicans* was analysed (Fig. 4D1). It is interesting that **7a-g** compounds have better affinity energy (ΔG) (-10.38, -10.35, -10.33, -10.40, -9.94, -10.25 and -10.49 *kcal/mol*, respectively), compared to fluconazole (-8.33 *kcal/mol*) as shown in Figure 3. This binding affinity is influenced by the number of ligand-receptor interactions and as show in Figure 4, **7g** has a higher number of interactions than fluconazole, which is reflected in its ΔG. However, another factor that is involved in biological activity is interactions with specific amino acids or cofactor.

In this sense, it should be noted that azoles that inhibit CYP51 activity interact with iron from the heme prosthetic group.[12] Likewise, the active site of CYP51 is formed by the amino acids of Glu116-Pro135, Leu220-Gly227, Tyr243-Ile253, Ile297-Ser314, Met374-Arg381 and Asp504-Glu514.[13] Figure 4D2 show that fluconazole binds with π-alkyl interactions with Ile131, Ile304, Leu376 and with the iron of the heme group (distance 3.77 Å), in the same way it bind to the hem group by π-donor H-bond, while with Tyr132 it binds with a H-bond and by π-π stacking.

Figure 4D3 shows the binding mode of the **7g** ligand, which is the compound with the best antifungal activity in the set under study, as reported in Figure 1. **7g** interacts with Tyr118 by π-π stacking and with Try132 with a H-bond, while with Ile131, Leu376,

His377, Phe380, Met508 and Val509 with π-alkyl interactions, with Gly307 through a C-H bond. The **7g** thiophene ring is bound with the heme group by π-alkyl interactions, while the thiazole ring forms a H-bond, it also interacts with the iron of the heme group with the pyrazole ring by π-alkyl interaction (distance 5.32 Å).

Although both compounds interact with both the heme group and iron, the interaction presented by fluconazole occurs at a more favourable distance than that of the **7g** molecule; possibly resulting in its increased antifungal activity. However, **7g** molecules; possibly resulting in its increased antifungal activity. However, **7g**, as it presents a good biological activity, serves as a template for structural modifications and thus increases its antifungal potential. Also, thanks to its structure, it can reduce the toxicity of azoles used in clinical therapy.

Table S3. Molecular docking methods selected by re-docking for RMSD value less than 2 Å			
Protein	**Scoring Function**	**Search Algorithm**	**RMSD**
PBP2a-1MWT *S. aureus*	MoldDock Score	MoldDock SE	1.24
PBP4-2EX8 *E. coli*	MoldDock Score	MoldDock Optimaizer	1.63
PBP4a-1W5D *B. subtillis*	MoldDock Score	MoldDock Optimaizer	1.84
CYP51-5TZ1 *C. albicans*	MoldDock Score GRID	MoldDock SE	1.57

A) RMSD 1.24 Å   B) RMSD 1.63 Å

C) RMSD 1.84 Å   D) RMSD 1.57Å
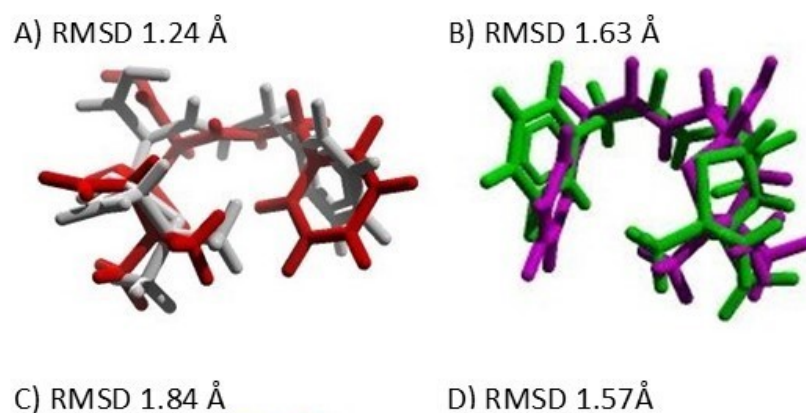
Figure S2. A) Overlapping of the co-crystallized ligand (white) and re-docked ligand (red) of the PBP2 protein of *S. aureus*. B) Overlapping of the co-crystallized ligand (green) and re-docked ligand (purple) of the PBP4 protein of *E. coli*. C) Overlapping of the co-crystallized ligand (yellow) and re-docked ligand (red) of the PBP4a protein of *B. subtillis*. D) Overlapping of the co-crystallized ligand (green) and re-docked ligand (blue) of the Sterol 14α-demethylase protein of *C. albicans*.

4. NMR Spectra

7a

168.32
159.49
145.54
139.17
134.64
130.11
129.31
128.33
127.93
127.51
119.13
116.72
114.50
102.00
55.64

OCH₃

7a

12.182

8.915
8.310
8.290
8.269
8.123
8.102
7.960
7.940
7.837
7.829
7.711
7.677
7.664
7.575
7.556
7.536
7.403
7.385
7.367
7.260
7.249
7.239

NO$_2$

7b

1.00

1.02
3.04
2.06
1.92
0.98
1.99
2.07
1.07
1.03

12 11 10 9 8 7 6 5 4 3 2 1 ppm

S22

7b

8.928
8.383
7.955
7.939
7.889
7.843
7.658
7.542
7.371
7.321
7.274
7.251
7.233

7c

1.00
1.01
5.07
1.00
2.00
0.83
1.09
2.99

11   10   9   8   7   6   5   4   3   2   1   ppm

7c

S25

7d
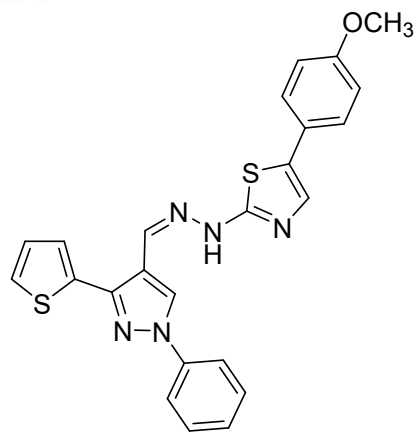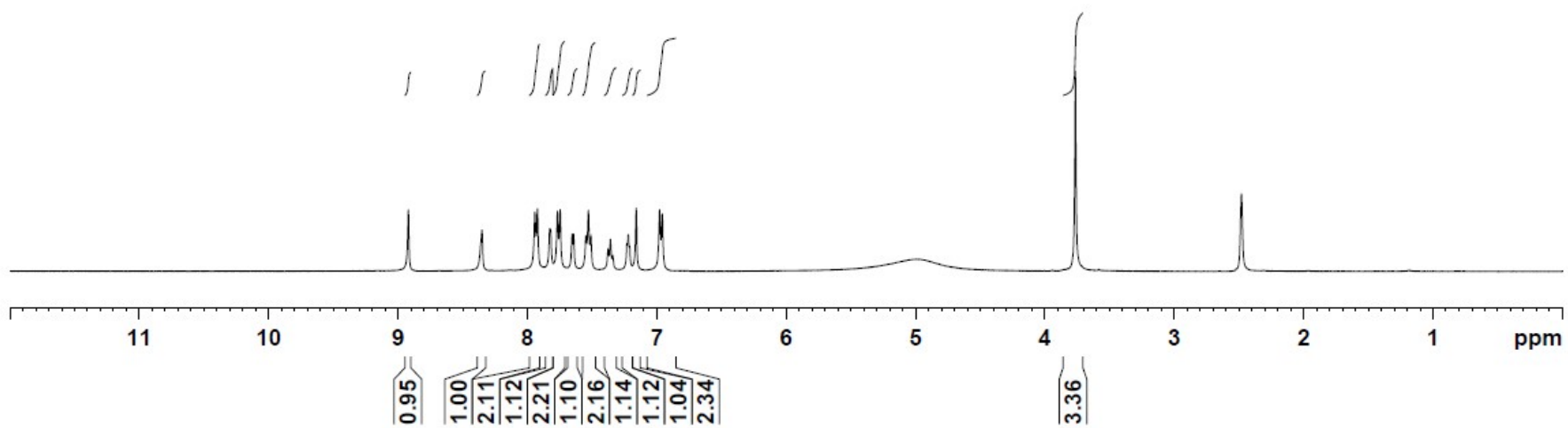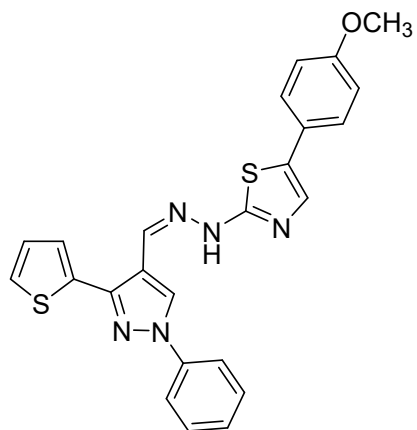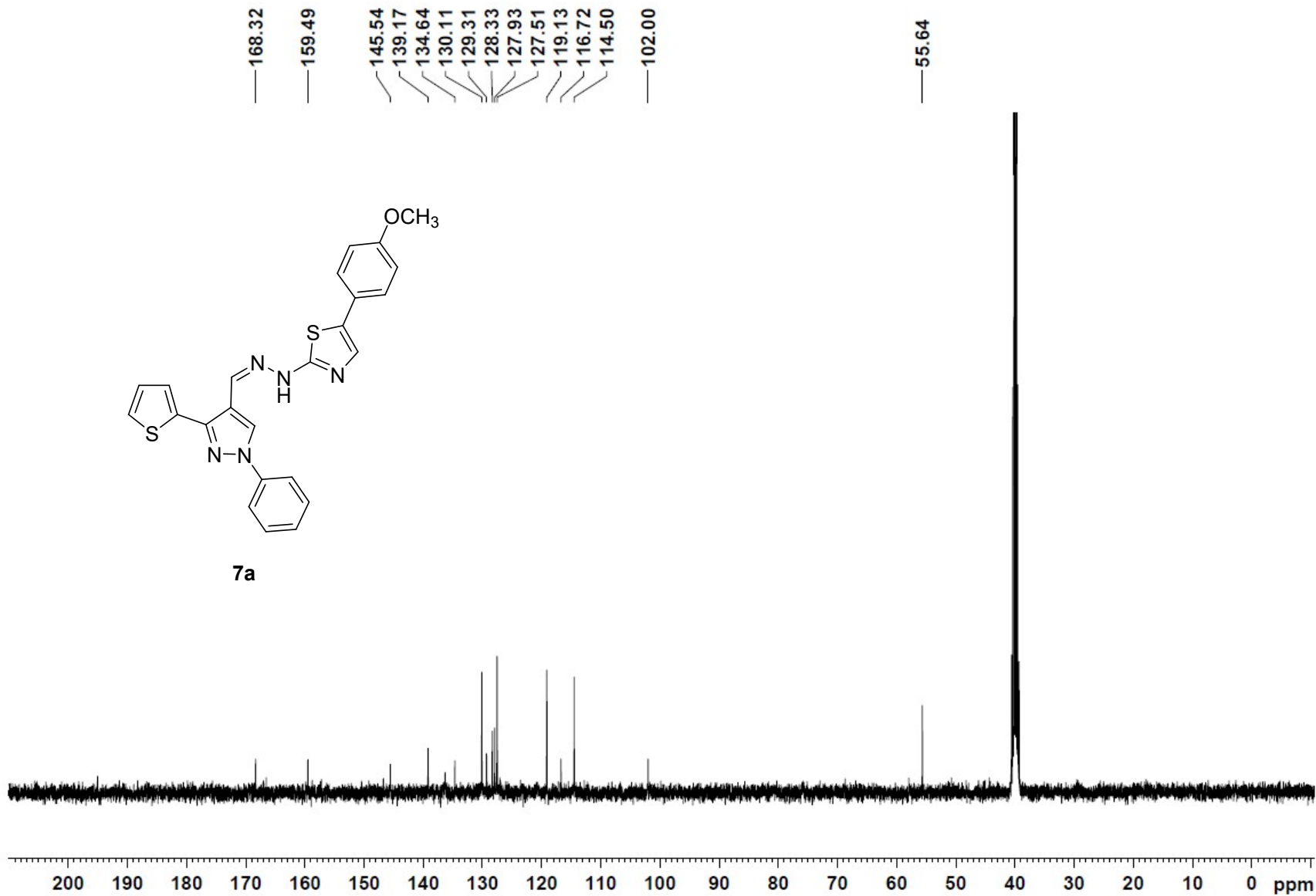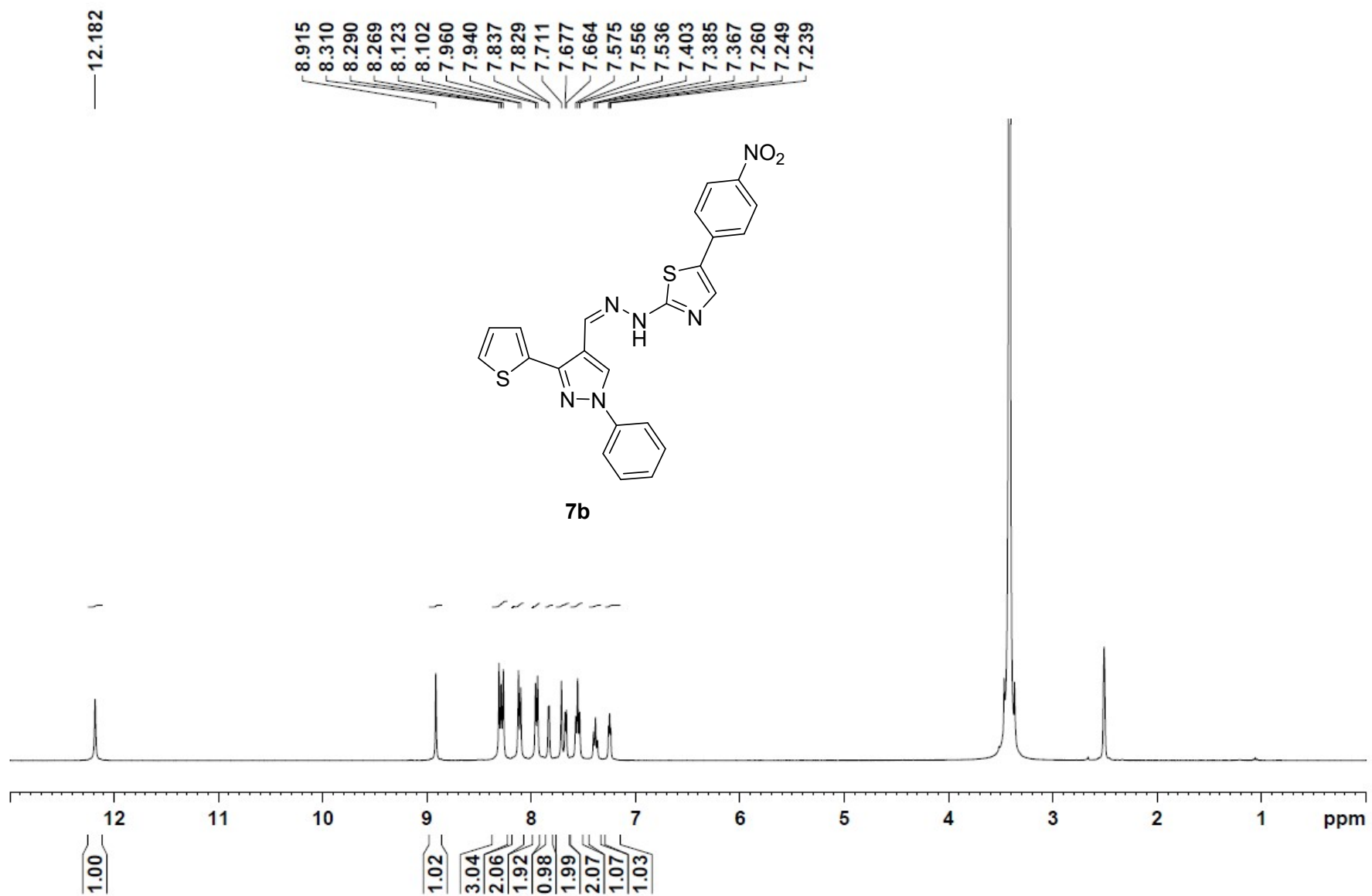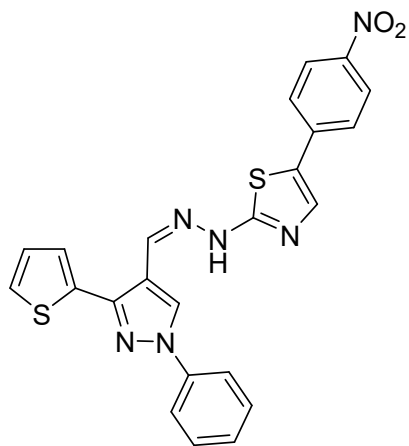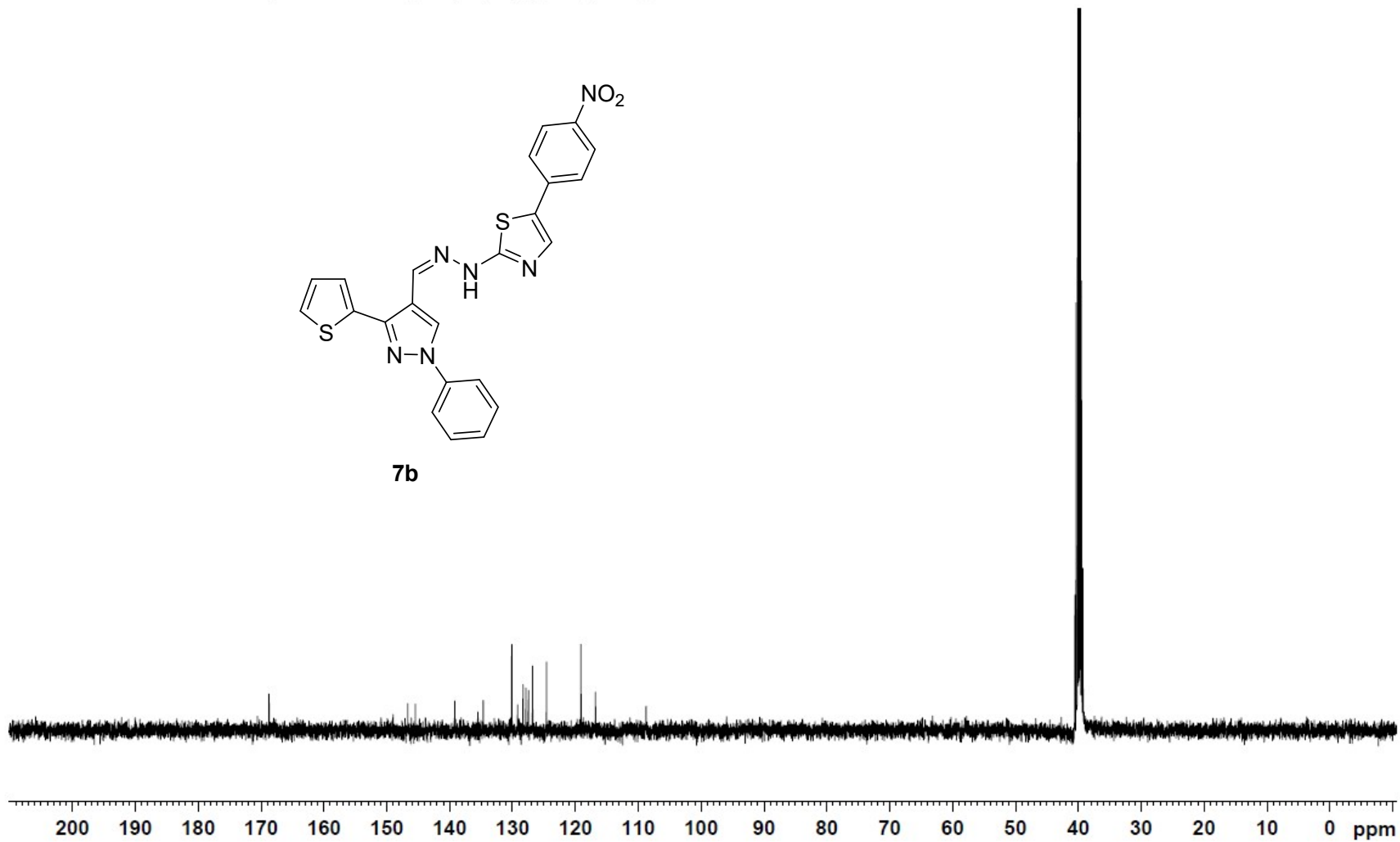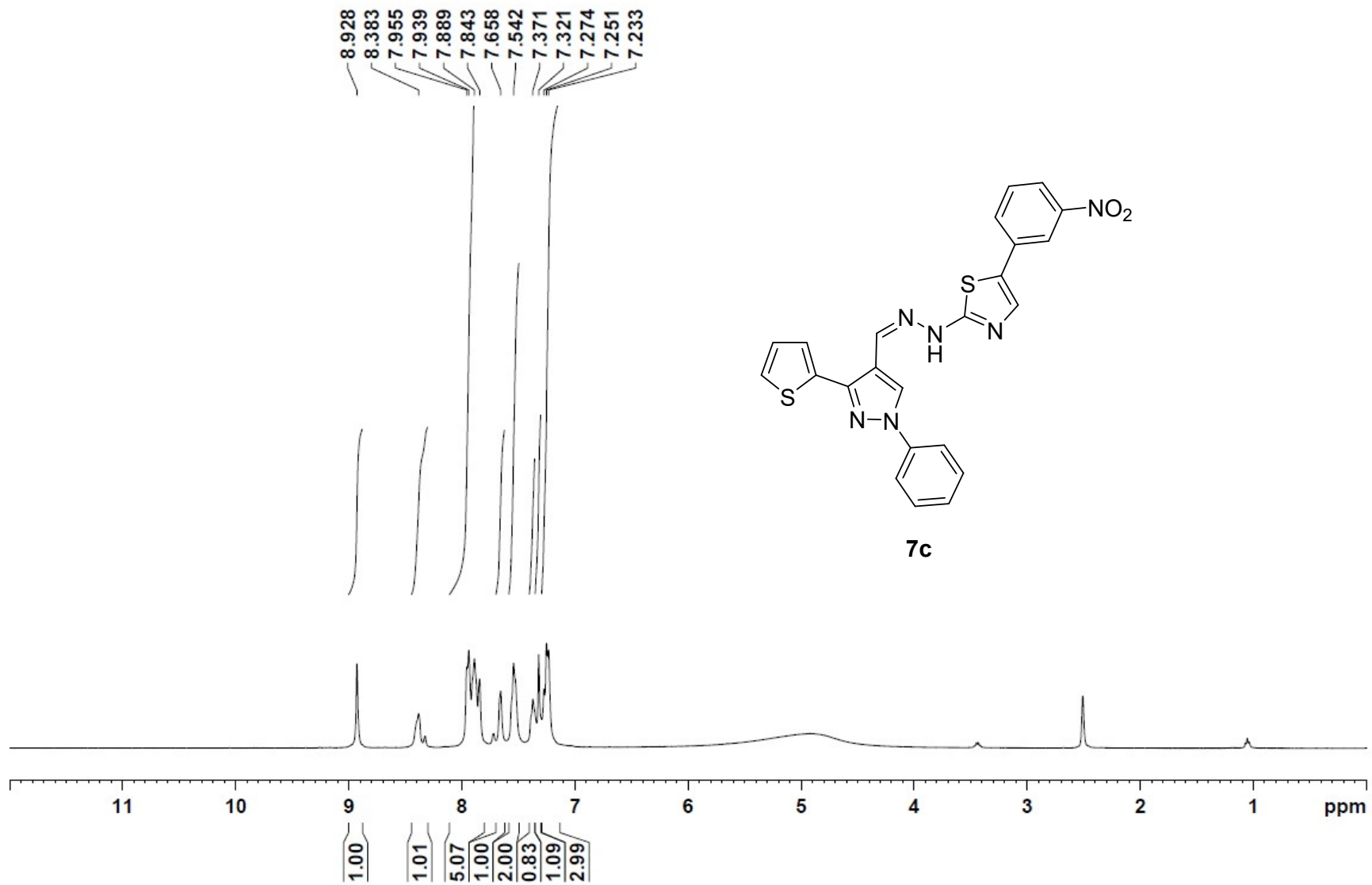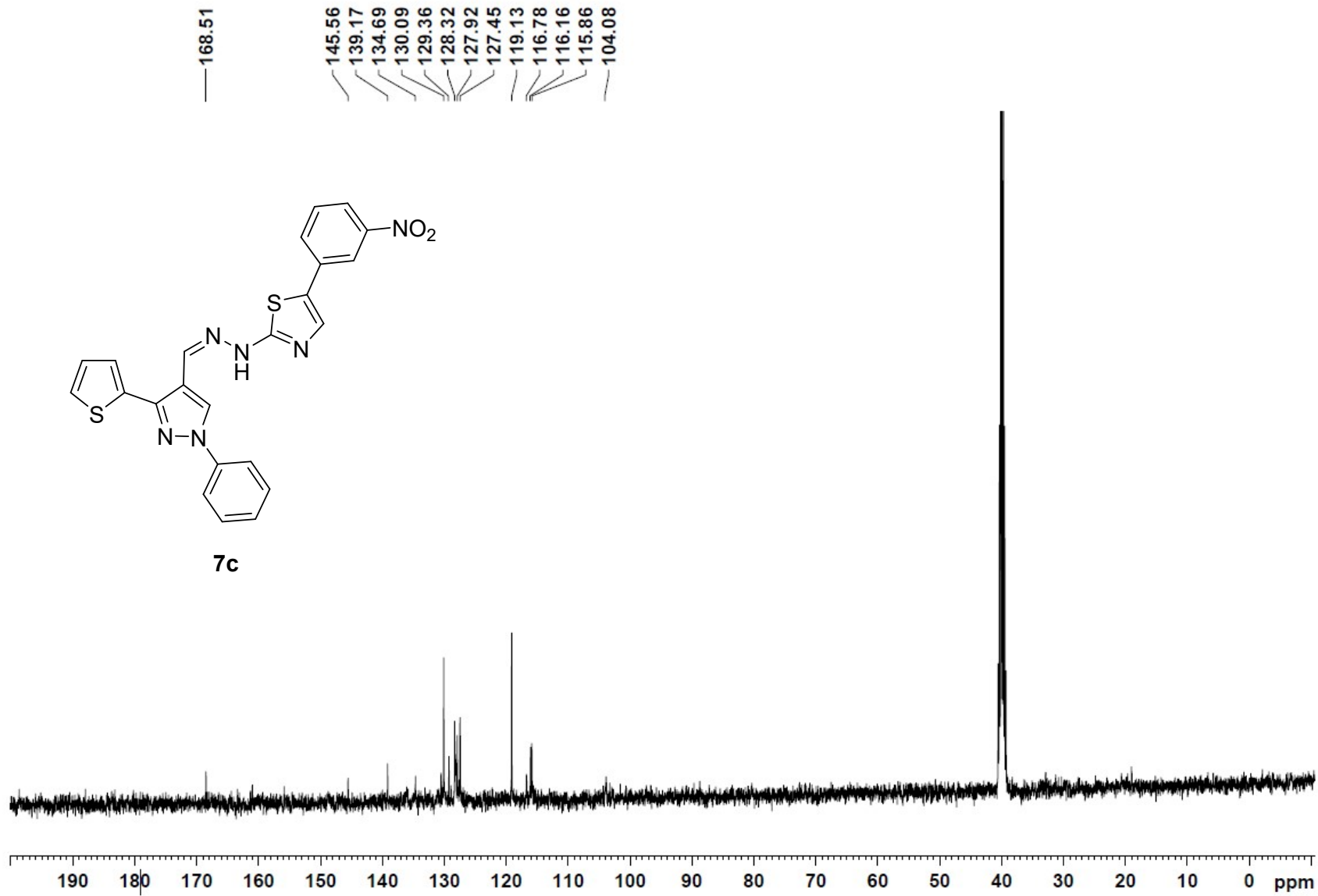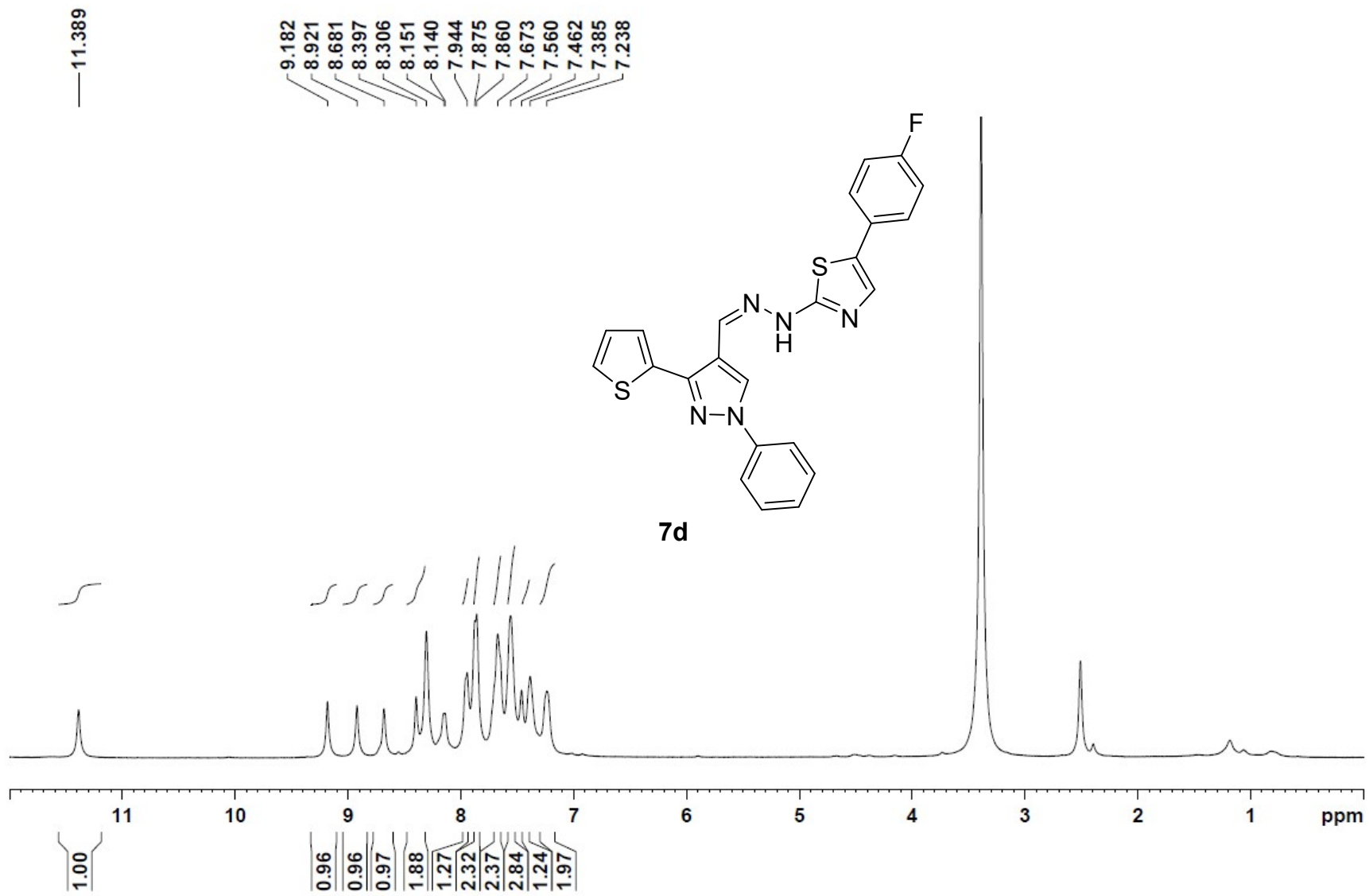
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148.75
148.59
139.21
136.65
135.43
135.00
134.72
134.38
132.04
130.68
130.18
130.10
129.27
128.50
128.31
127.90
127.58
127.44
126.80
125.23
122.48
120.42
119.11
118.93
117.31
116.82
106.58

7d

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

8.939
8.346
7.976
7.902
7.865
7.729
7.687
7.567
7.495
7.415
7.258

7e

0.97
1.02
5.13
8.01
1.06

11 10 9 8 7 6 5 4 3 2 1 ppm

168.70

145.48
135.48
134.72
130.11
129.12
128.90
128.33
127.89
127.76
127.44
119.14
116.86
104.75

7e

7f

167.80

145.00
138.65
131.13
130.45
129.61
127.68
127.51
127.03
126.89
121.75
118.64
116.28

7f

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

S31

8.921
8.457
7.920
7.812
7.695
7.636
7.506
7.336
7.255
7.209
2.277

CH₃

7g

1.11
1.29
3.17
1.01
3.19
1.87
1.22
3.01
3.00

11 10 9 8 7 6 5 4 3 2 1 ppm

168.47 147.71 145.70 139.11 138.11 137.74 134.54 130.46 130.08 129.73 129.45 129.32 128.34 128.22 127.98 127.52 126.22 119.14 116.48 103.50
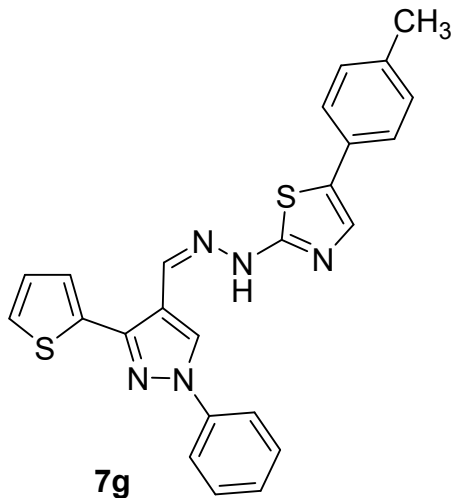
21.30
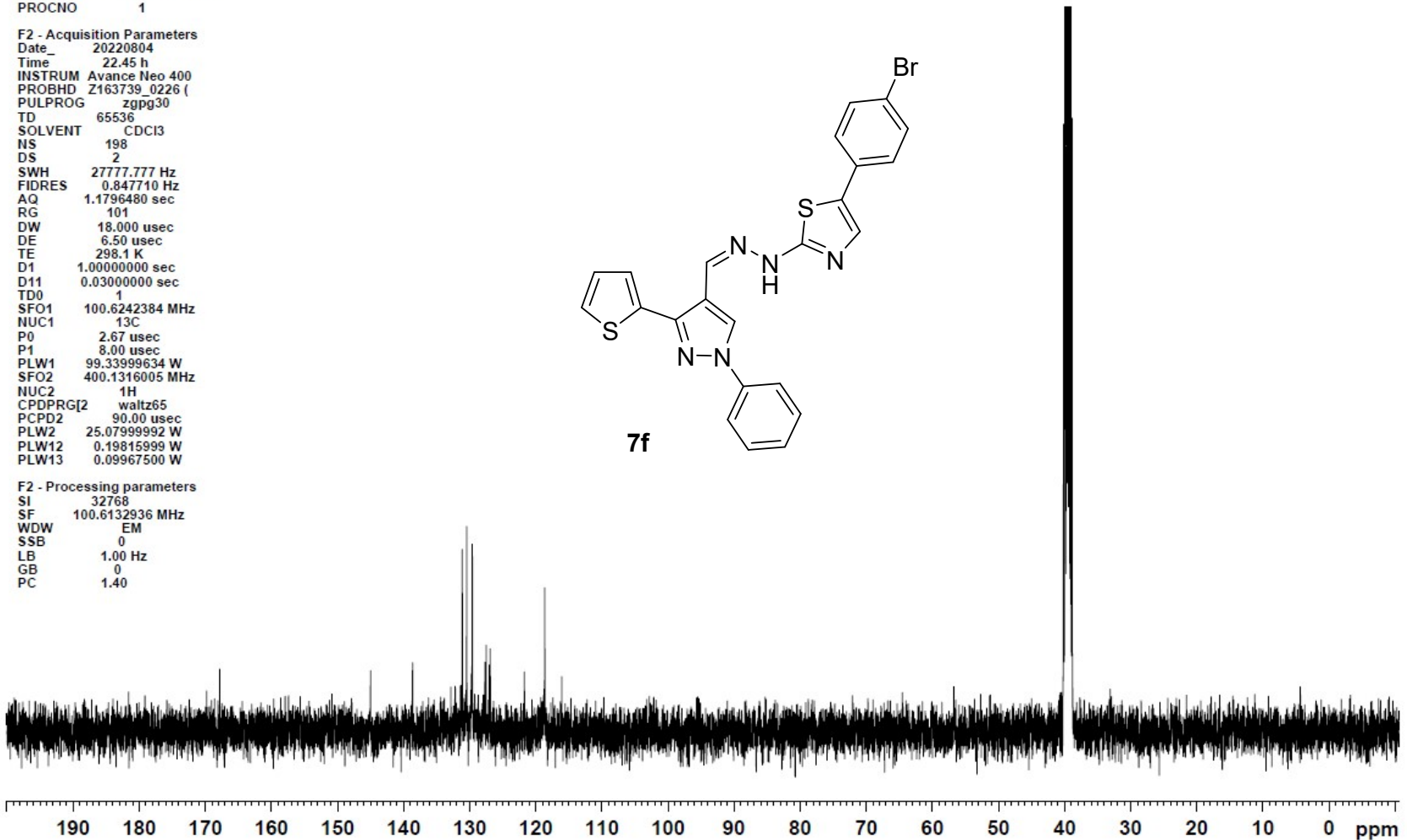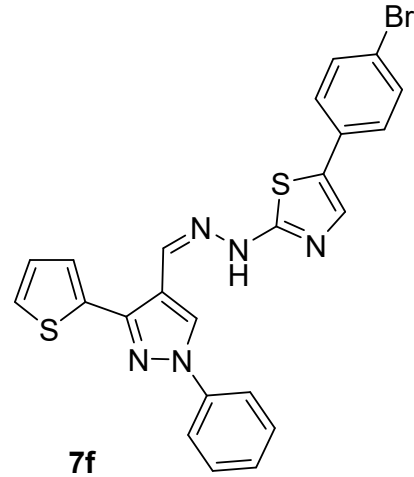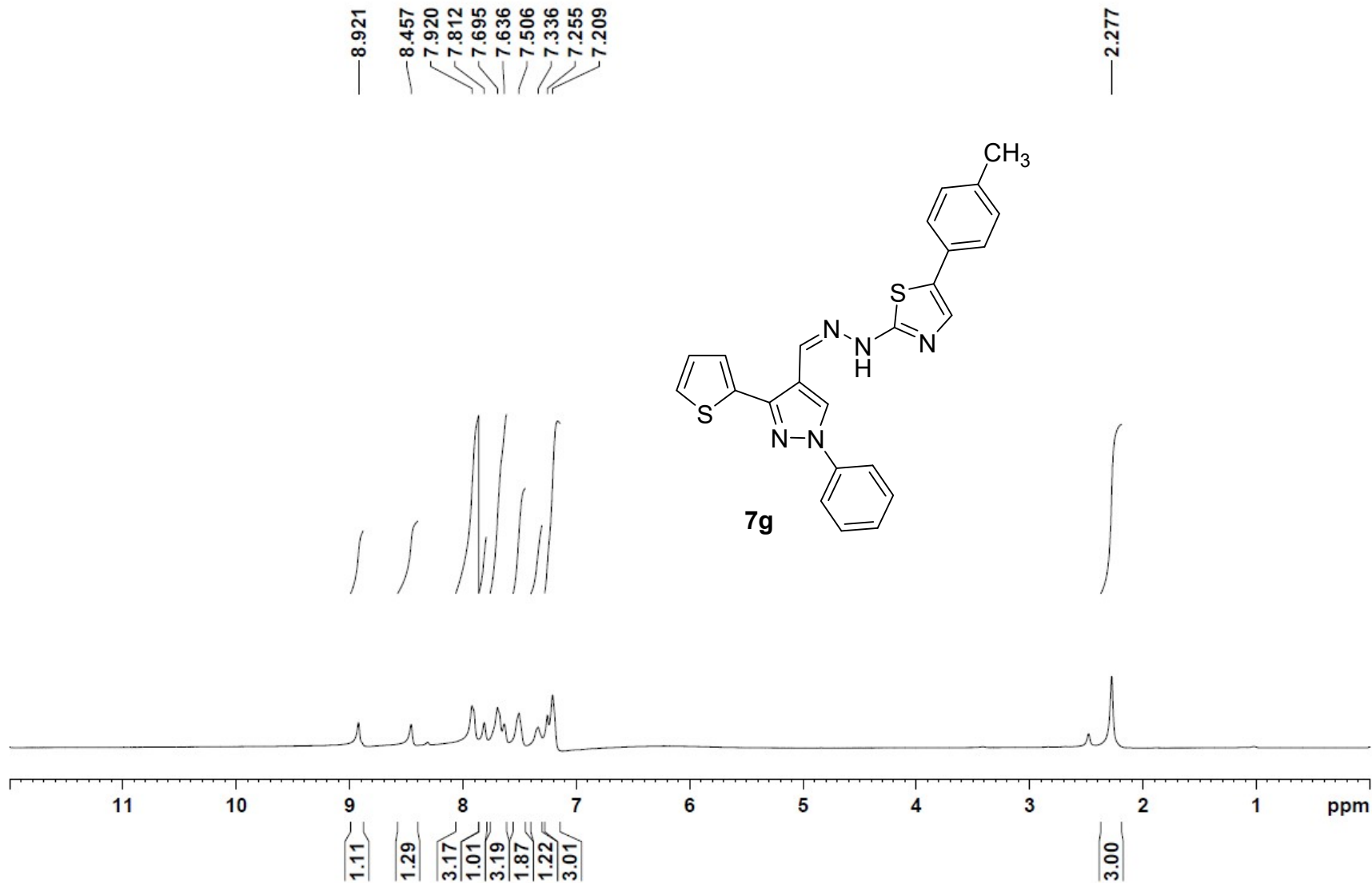
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NUC2   1H
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PLW12   0.26944000 W
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PC   1.40

7g

S33

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