Macedonian Journal of Chemistry and Chemical Engineering, Vol. **42**, No. 1, pp. 1–12 (2023)

Supplementary material

BIOACTIVITY AND QUANTUM CHEMICAL CALCULATIONS OF A NEW COUMARINE DERIVATIVE AS A STRONG ANTIOXIDANT, ANTIMICROBIAL AND ANTI-CANCER SUBSTANCE

Pelin Koparir

Firat University, Vocational School, Department of Forensics Chemistry, 23169 Elazig, Turkey
mpelin23@hotmail.com

Table S1: Selected optimized geometric parameters of the title compound in the ground stat

Bond lengths (Å)	B3LYP/6-311G (d, p)	Torsional angles (º)	B3LYP/6-311G (d, p)
S1-C1	1.764	C2-N2-N1-C1	-0.048
S1-C9	1.837	C1-N3-C2-N2	-0.351
N2-C2	1.315	N2-N1-C1-S1	-179.73
N2-N1	1.372	C2-N3-C1-S1	179.89
C1-N1	1.310	C12-O1-C13-C18	0.022
N3-C1	1.372	C12-O1-C13-C14	-179.95
N3-C2	1.385	C17-C18-C13-O1	-179.95
N3-C7	1.465	C13-O1-C12-O2	179.95
C7-C8	1.529	C10-C11-C12-O2	-179.96
O1-C13	1.362		
O1-C12	1.367		
O2-C12	1.201		
C13-C18	1.404		
C12-C11	1.455		
C10-C11	1.352		
Bond angles (º)		**Bond angles (º)**	
C1-N1-N2	107.04	O2-C12-C11	126.01
C2-N2-N1	108.22	O1-C12-C11	115.93
C1-N3-C2	103.62	C13-C14-C15	120.44
C1-N3-C7	126.54	C14-C15-C16	118.52
C2-N3-C7	129.74	C15-C16-C17	121.01
N2-C2-N3	109.84	C14-C13-C18	121.56
N1-C1-N3	111.26	C13-C18-C17	117.39
N1-C1-S1	126.31	C16-C17-C18	121.06
N3-C1-S1	122.42	C2-C3-S2	117.80
C12-O1-C13	122.3	C4-C3-C2	131.50
O2-C12-O1	118.04		

Table S2: Experimental and calculated chemical shifts (ppm) of ^{1}H-NMR for the title compound

H number	**Experimental (E)**	**Theoretical DMS phase (T)**	$\frac{E-T}{E}$**X100**
4	7.85	7.54	3.94
5	7.27	7.45	-2.47
6	7.27	7.35	-1.10
7	4.09	4.14*	-2.93
8	1.17	1.59*	-5.12
9	4.62	4.44*	3.89
11	6.32	6.25	1.10
14	7.57	7.45	1.58
16	7.85	7.69	2.03
17	7.27	7.58	-4.26
19	3.39	3.09*	8.84

*: The average value

Table S3: Experimental and calculated chemical shifts (ppm) of ^{13}C-NMR for the title compound

C number	**Experimental (E)**	**Theoretical DMS phase (T)**	$\frac{E-T}{E}$**X100**
1	149.4	158.5	-6.09
2	150.3	158.8	-5.65
3	149.6	141.9	5.14
4	125.9	131.8	-4.68
5	128.0	133.7	-4.45
6	128.7	140.1	-8.85
7	33.8	35.9	-6.21
8	15.3	16.6	-8.49
9	40.2	42.0	-4.47
10	151.4	159.9	-5.26
11	114.4	115.9	-1.31
12	160.1	165.2	-3.18
13	153.9	162.4	-5.52
14	117.2	123.2	-5.11
15	143.5	154.3	-7.53
16	128.0	131.9	-3.04
17	125.4	130.1	-3.74
18	115.6	122.1	-5.62
19	21.2	22.4	-5.66

Table S4: Comparison of the observed and calculated vibrational spectra of the title compound

Assignments With TED	Unscaled Frequencies (6-311(d,p)) B3LYP	FT-IR (cm^{-1}) With KBr	Assignments With TED	Unscaled Frequencies (6-311(d,p)) B3LYP	FT-IR (cm^{-1}) With KBr
υasC16H	3043		υC13C14	1559,1239,1130	
υsC17H	3065	3069	υC16C17	1711, 1239	1217
υasC14H	3062		βC9C10C11	1491,941,586	1501
υC11H	3078		βN1C1N3	1600	1605
υsC6H	3144	3081	βC16C17H	1601,1372,1243	
υasC5H	3076		βS2C6H	1432,1151	
υsC4H	3096		βS1C9H	1261,984	
υasC19H	2987		βO1C12O2	591,586	
υasC9H	2986		βC1N2N1	1463,1064,1004	
υasC7H	2997		βO1C13C14	591,586	
υsC8H	2917	2937	βN2N1C1	1064	
υsC19H	2906		βC8C7N3	1144,446	
υasC8H	2983		ωC16H	1383	1366
υC12O2	1748	1717	ωC5H	1111	1155
υC10C11	1759,1669		ωC19H	1060	1086
υN1C1	1600,1459	1439	αC18C17C16H	1047	
υN2C2	1714,1628,1461	1474	αO1C12C11H	984,885,748,749	897
υC5C8	1628,1536,1151		αC1S1C9H	1361,1261,984	
υC3C4	1714,1628,1536	1705	αN3C7C8H	1550,1212,1144	
υC1N3	1483		αN3C1N1N2	791,744	
υC13O1	1496,1372,1270	1266	αS2C6C5C4	999,563	
υC12O1	1302,1300,1280,941	938	δO2C11O1C12	913,798	
υN1N2	1097	1134	δC19C16C14C15	1112,634	1155
υN3C7	709		δC3N3N2C2	800	808
υS2C6	878,775,668	740	δC11C9C18C10	984,583	740
υS1C1	563,539	560	δO2C18C14C13	942,559	
υS1C9	838,820,586				

υ,stretching; β, bending; ω, in plane bending ; δ, out of plane bending; α, torsional; s, symmetric; as, asymmetric.

Table S5: Global reactivity descriptors for the title compound (V)

Parameters	**B3LYP/6-311G (d, p)**
E_{HOMO} (eV)	-6.0653
E_{LUMO} (eV)	-2.0081
$\Delta E = E_{LUMO} - E_{HOMO}$ (eV)	4.0572
I (eV)	6.0653
χ (eV)	4.0367
η (eV)	2.0286
S (eV^{-1})	0.2464

Table S6: Thermodynamic properties of the title compound.

Parameters	**B3LYP/6-311G (d, p)**
Zero-point vibrational energy (kcal/mol^{-1})	204.64084
Rotational constants (GHz)	0.41215
	0.06502
	0.05662
Rotational temperatures (Kelvin)	0.01978
	0.00312
	0.00272
Entropy (cal mol^{-1} K^{-1})	
Translational	43.721
Rotational	36.603
Vibrational	95.712

Table S7: Antimicrobial activities of test compound. Minimum inhibitory concentration values (MIC, μg/mL)

Groups	**Bacteria**						**Fungi**	
	E.coli ATCC 25922	***S.aureus*** ATCC 29213	***P.aeruginosa*** ATCC 27853	***K.pneumoniae*** ATCC 13883	***B.cereus*** ATCC 11778	***E.faecalis*** ATCC 29212	***C.albicans*** ATCC 10231	***C.tropicalis*** DSM 11953
Test Compound	0,081	0,064	0,571	0,554	0,069	0,585	0,041	0,071
Fluconazole (Standart for fungi)	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	0.5	0,025
Ciproflaxin (Standart for bacteria)	0,007	0,025	0,05	0,25	0,05	0,12	N.T.	N.T.

N.T.= Not Tested

Table S8: TAS, TOS and OSI values of the title compound

	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
Test Compound	19.896±0.712	6.999±0.518	0.037±0.654

Table S9: The IC_{50} (μM±SD) values of test compound on different cell lines (24 hour).

Groups	HUVEC Human umbilical vein endothelial cell	MCF-7 Human breast adenocarcinoma cell	MKN-45 Human gastric cancer cell line	Selectivity analysis
Test Compound	>100	7.06 ± 0.48	9.79± 1.91	MCF-7 selective
Doxorubicin (Standart)	>10	4.7± 0.16	4,16 ± 0,25	

IC_{50}: indicate the inhibitory concentration capable of reducing cell viability by 50%. Data are represented as the mean of three independent experiments ±standard error (SE). IC_{50} > 100.0 μM are considered as not active. Doxorubicin is positive control.

Table S10: (%) DPPH· effect of synthesized compound

Groups	**50 µg/mL**	**100 µg/mL**	**250 µg/mL**
Test Compound	37,82	48,71	51,83
BHT	30,43	41,45	46,66

Table S11: Molecular docking binding scores of title compound within the some macromolecules

	Visualization Results of Docking			**Autodock**		**Vina**
Pdb ID	**H-bonds**	**Pi-Pi Stacking**	**Pi Cation**	**Estimated Inhibition Constant, Ki**	**Best Docking Score**	**Best Docking Score**
1BQB	ARG200	HIS144 TYR190	ARG200	9.75 μM	-6.84	-8.1
6TZ6	A:TRP401 C:TYR97 C:PHE114	-	-	190.66 nM	-9.17	-9.5

μM: micromolar, nM : nanomolar, Docking Score: Estimated Free Energy of Binding (kcal/mol)

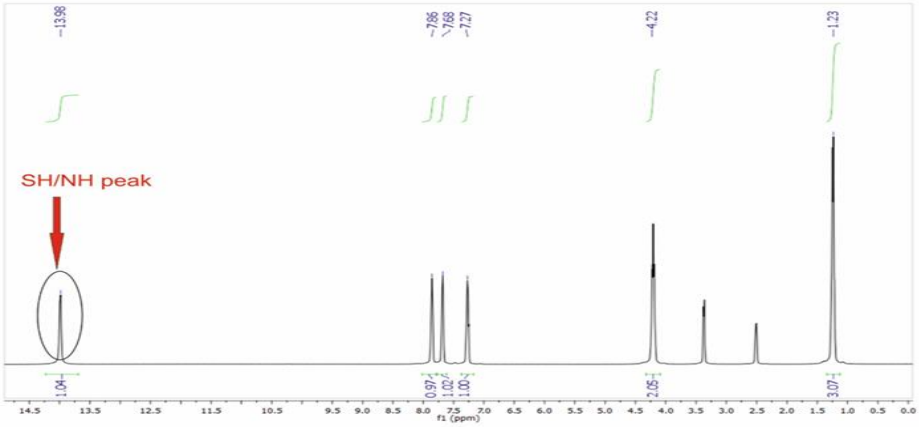

Figure S1: The ^{1}H-NMR spectrum of 4-Ethyl-5-(thiophene-2-yl)-4H-1,2,4-triazole-3-thiol (III)

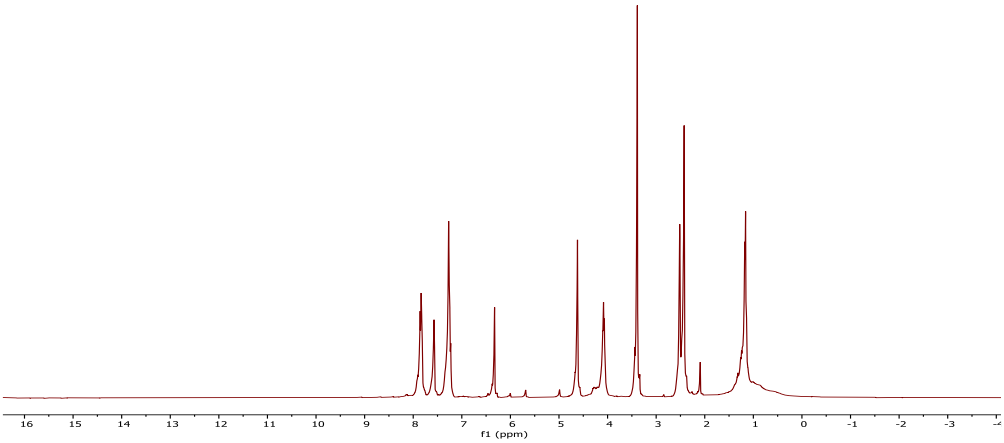

Figure S2: ^{1}H-NMR spectrum for the title compound (V)

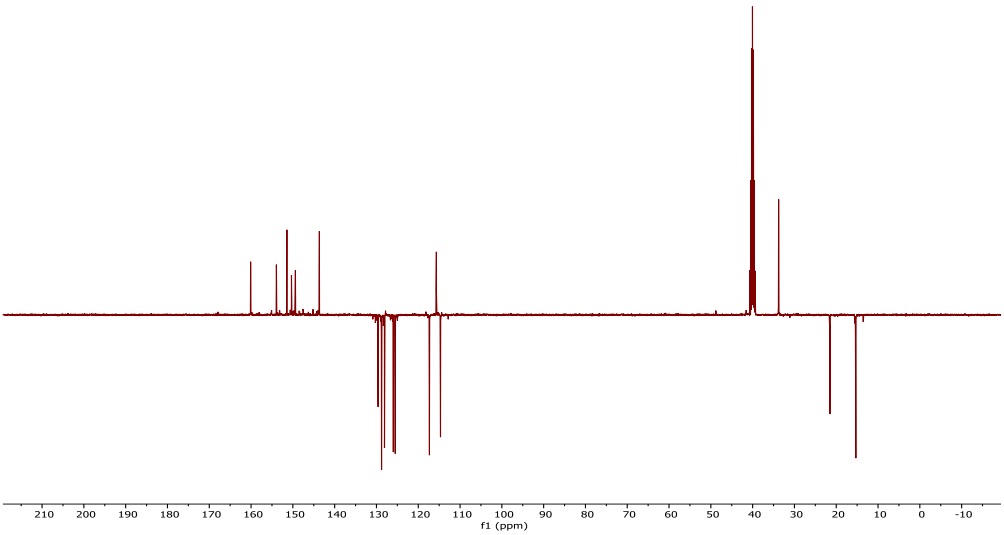

Figure S3: ^{13}C-NMR spectrum for the title compound (V).

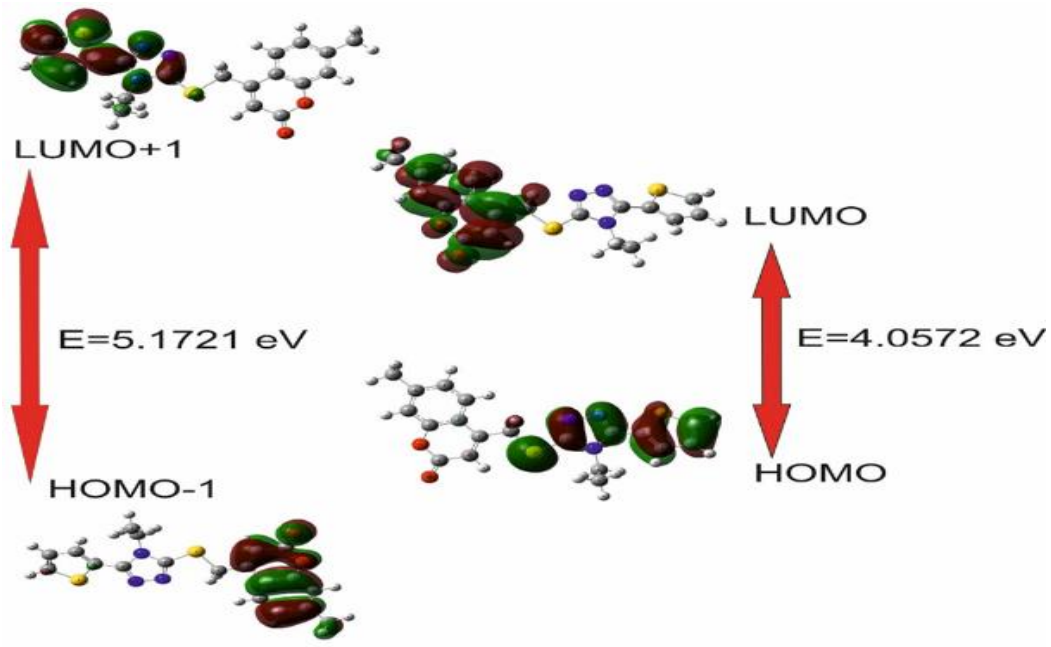

Figure S4: Molecular orbital surfaces and energy levels of the title compound (V)

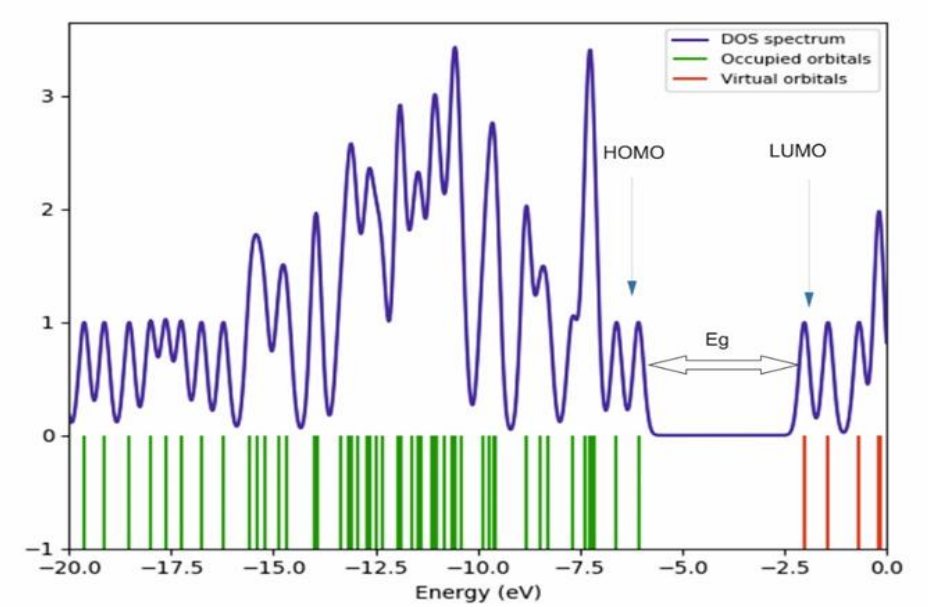

Figure S5: The density of states diagrams for the title compound (V)

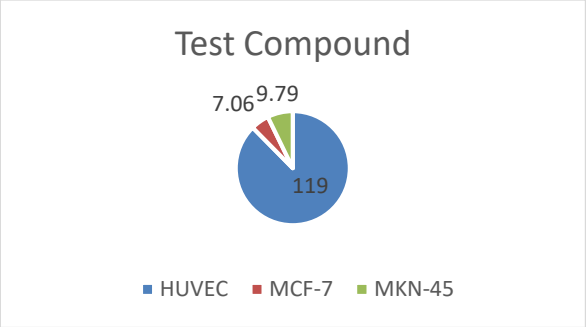

Figure S6: Comparison of IC_{50} values (µM±SD)) of the test compound on different cell lines

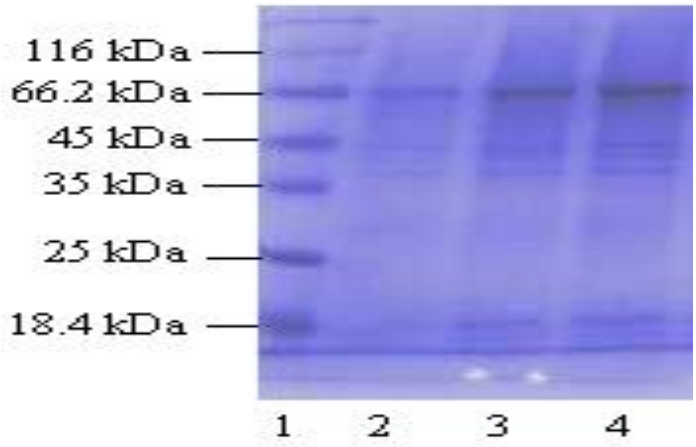

Figure S7: Results of Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) lines: (1) Marker; (2 and 3) DMSO; (4) Test Compound (24 h, 10 μM supernatant MCF-7 cancer cell extract with test compounds)

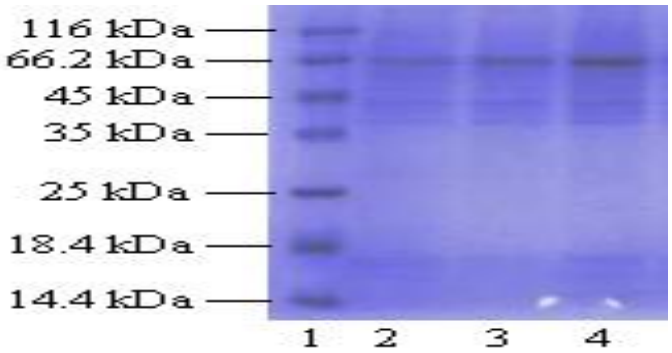

Figure S8: Results of Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) lines: (1) Marker; (2 and 3) DMSO; (4) Test Compound (24 h, 10 μM supernatant MKN-45 cancer cell extract with test compounds)

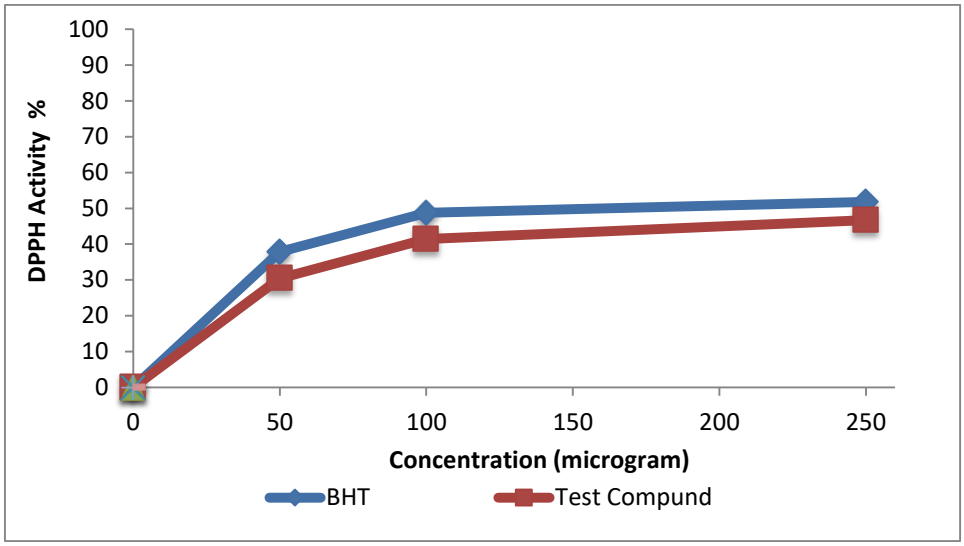

Figure S9: % DPPH activity of test compound