Unveiling the Multifaceted Bioactivity of Copper (II) Schiff Base Complexes: A Comprehensive Study on Antioxidant, Anti-bacterial, Anti-inflammatory, Enzyme Inhibition and Cytotoxic Potentials with DFT Insights

T. M. Dhanya[1], M. R. Prathapachandra Kurup[1], K.J. Rajimon[2], G. Anjali Krishna[1,3], K. V. Jibin[4], K. G. Raghu[5], Sachin Philip[1], K. M. Divya[1,6], Maria Augustine[1,7], and P. V. Mohanan[1,*]

[1]Department of Applied Chemistry, Cochin University of Science and Technology, Kochi 22, Kerala, India, Email: mohan@cusat.ac.in

[2]Department of Chemistry, St. Berchmans College, Changanacherry, Kerala, India,Email:rajimonkalambukattu@gmail.com

[3]Department of Science and Humanities, Mar Baselios Institute of Technology and Science, Nellimattom, Kothamangalam, Kerala, India, Email:anjalikrishna@mbits.ac.in

[4]Jr. HSST Chemistry, KMHSS Kuttoor, North Malappuram, India,

Email: jibinkchem@gmail.com

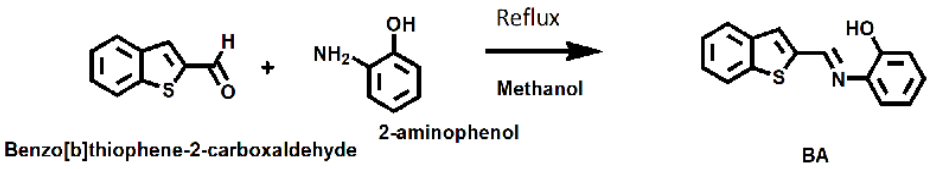
[5]Agro-Processing and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram, *Kerala, India, Email:raghukgopal@niist.res.in*

[6]Department of Chemistry, NSS College, Cherthala, Kerala, India, Email:divyadevuty2008@gmail.com

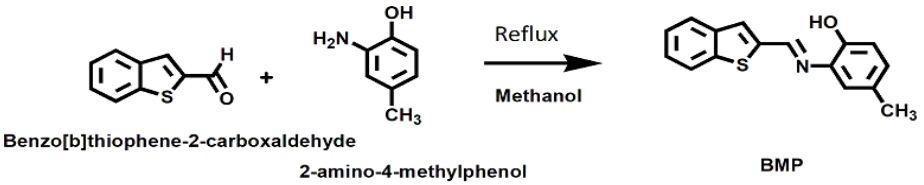
[7]Department of Chemistry, St. Paul's College, Kalamassery, Kerala, India,

Email:mariamartinjoe@gmail.com

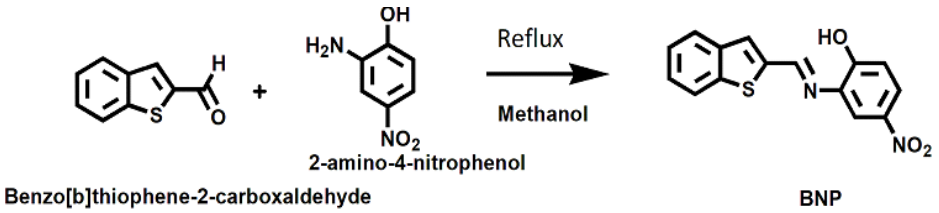
Scheme S1. The process involved in the synthesis of the Schiff base ligand BA.



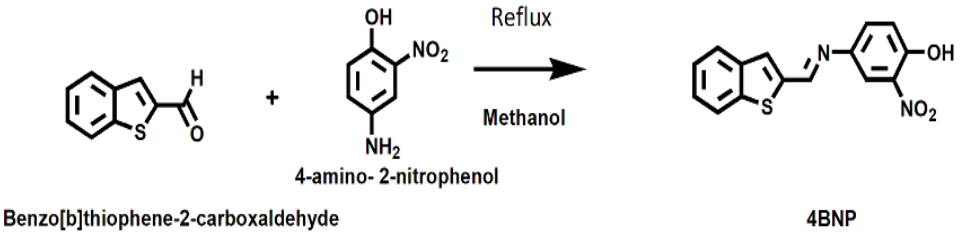
Scheme S2. The synthetic route involved in the formation of the Schiff base ligand BMP.



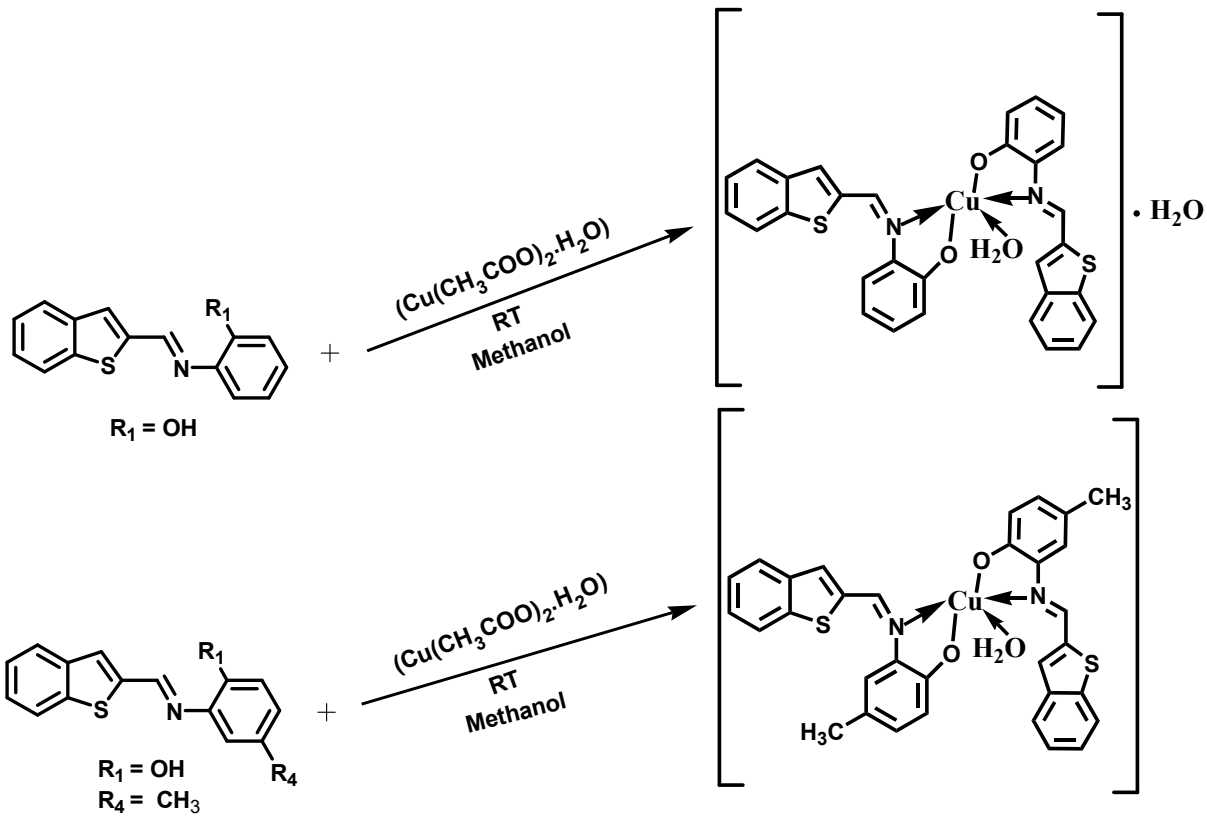
Scheme S3. The synthetic route involved in the formation of the Schiff base ligand BNP.



Scheme S4. The synthetic route involved in the formation of the Schiff base ligand 4BNP.



Scheme S5. The scheme shows the synthesis of Cu(II) complexes derived from the benzo[b]thiophene Schiff base ligand BA and BMP.


R1
S
N
R1 = OH
+
(Cu(CH3COO)2.H2O)
RT
Methanol
O
Cu
N
O
H2O
S
S
N
• H2O
R1
S
N
R1 = OH
R4 = CH3
R4
+
(Cu(CH3COO)2.H2O)
RT
Methanol
CH3
O
Cu
N
O
H2O
S
S
N
H3C

Scheme S6. The scheme shows the synthesis of Cu(II) complexes derived from the benzo[b]thiophene Schiff base ligand BNP and 4BNP.

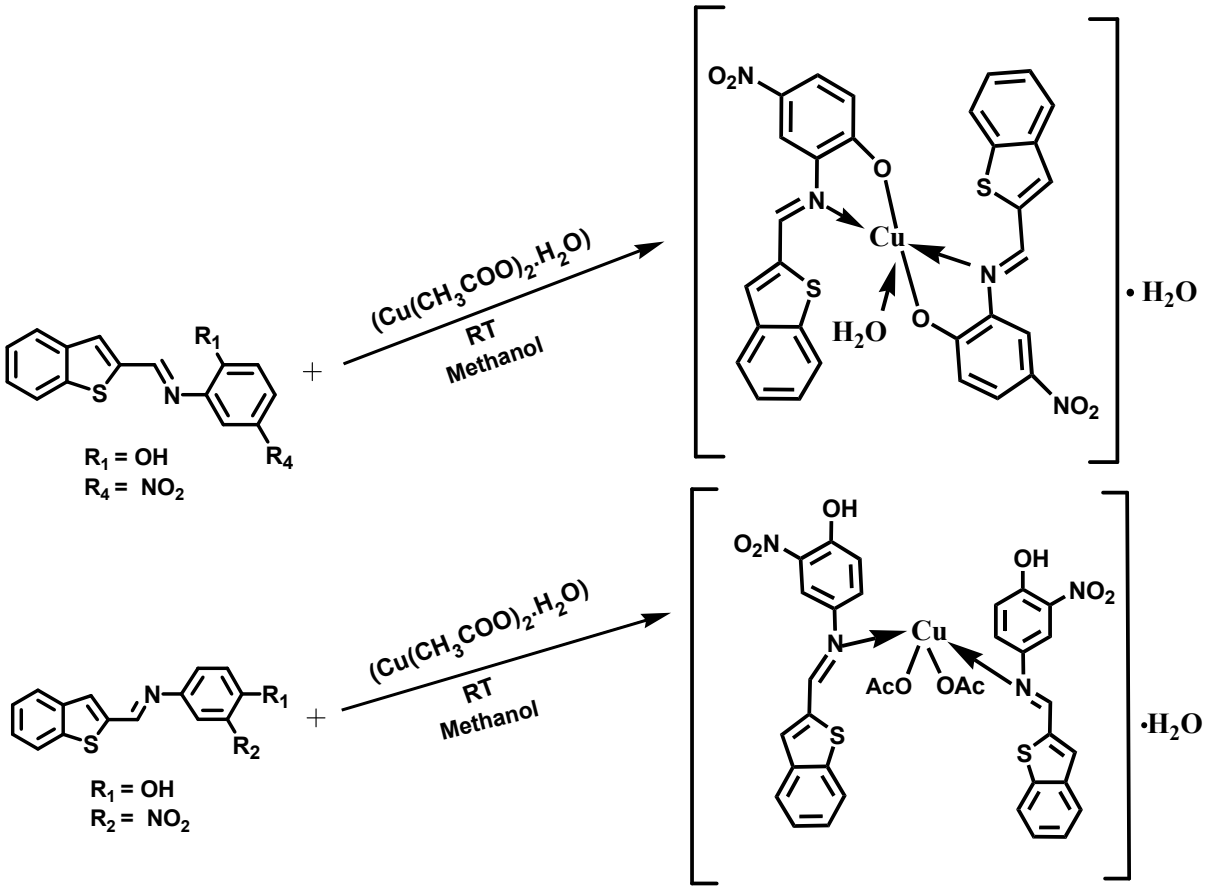


Fig. S1. The UV-visible absorption spectra of synthesized ligand incorporated metal complex BAC and BMPC.

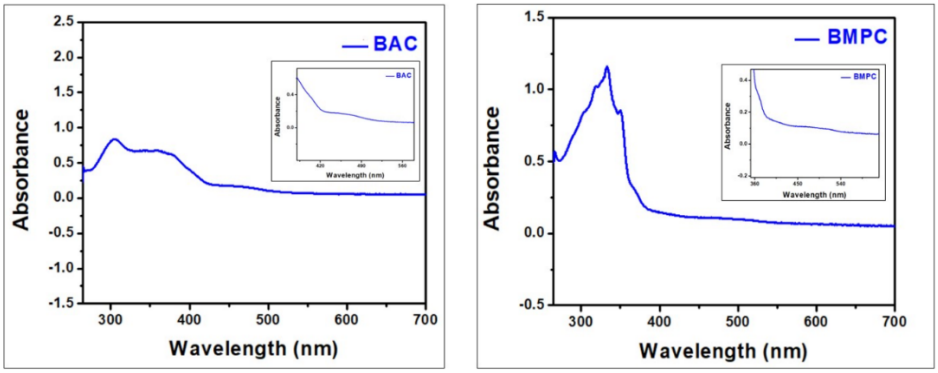


Fig. S2. The UV-visible absorption spectra of synthesized ligand incorporated metal complex BNPC and 4BNPC.

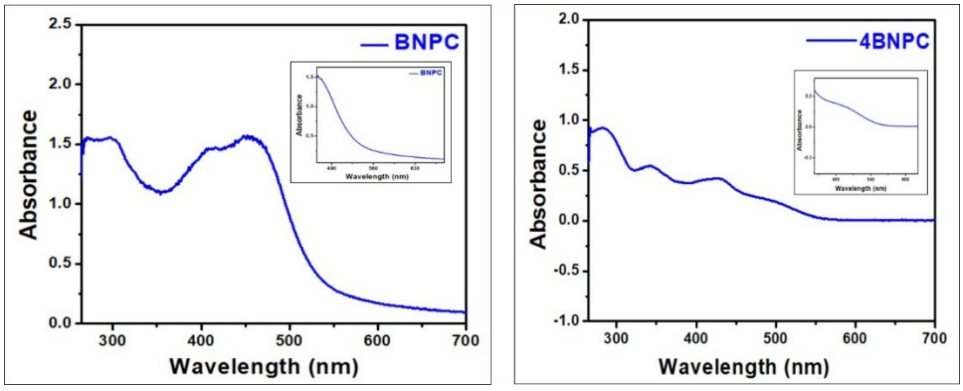


Fig.S3. IR spectra of the BA, BMP derived Cu(II) complex.

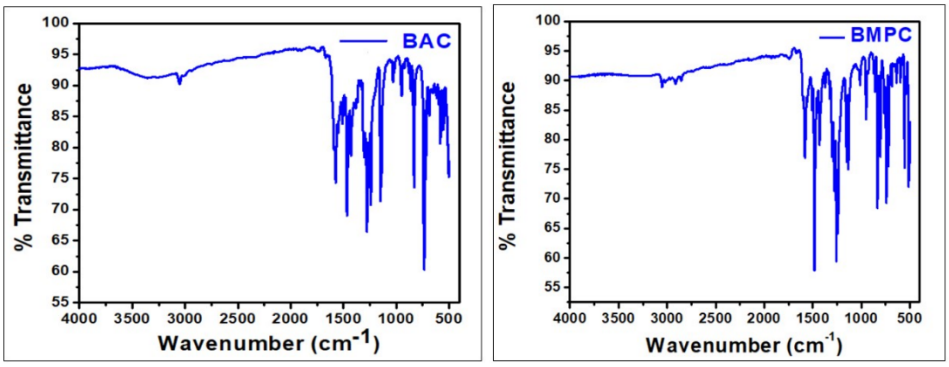


Fig.S4. IR spectra of the BNP, 4BNP derived Cu(II) complex.

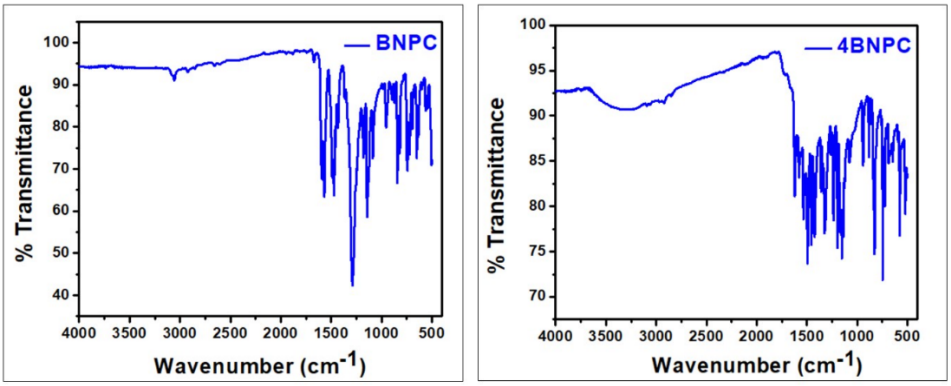


Fig.S5. TG-DTG graphs of Schiff base ligand BA, BMP incorporated metal complexes of Cu(II).

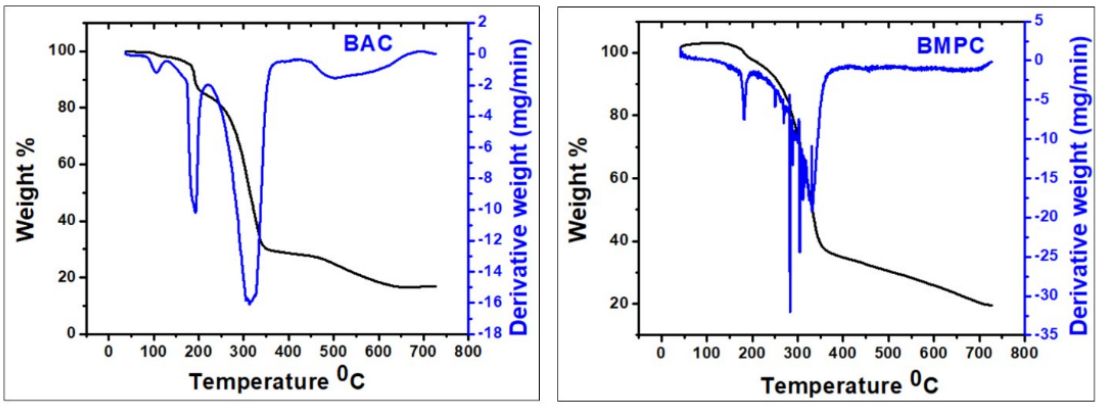


Fig.S6. TG-DTG graphs of Schiff base ligand BNP, 4BNP incorporated metal complexes of Cu(II).

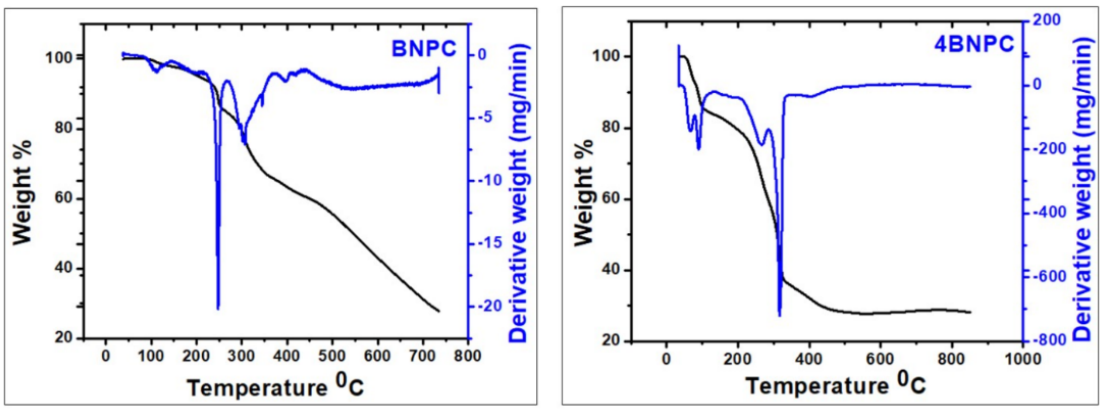


Fig.S7. The EPR spectrum of BAC in their polycrystalline state at 298 K.

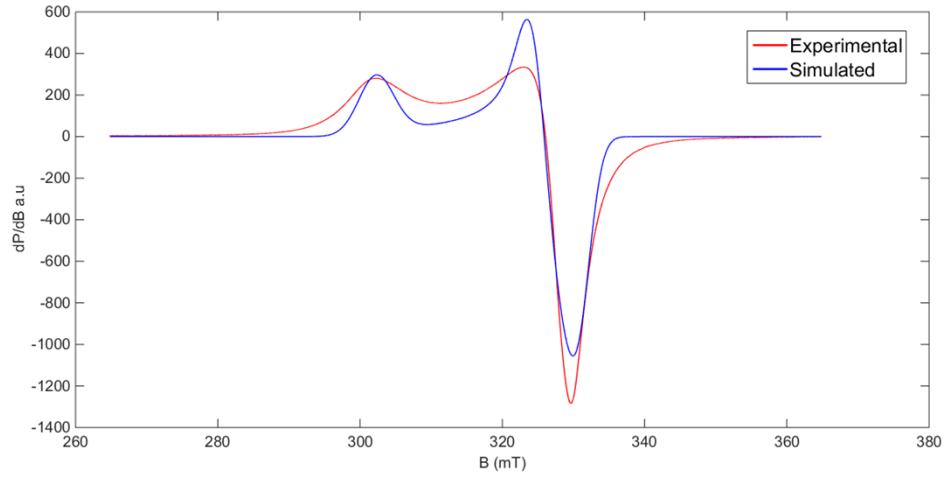


Fig.S8. The EPR spectrum of BAC in DMF as solvent at 77 K.

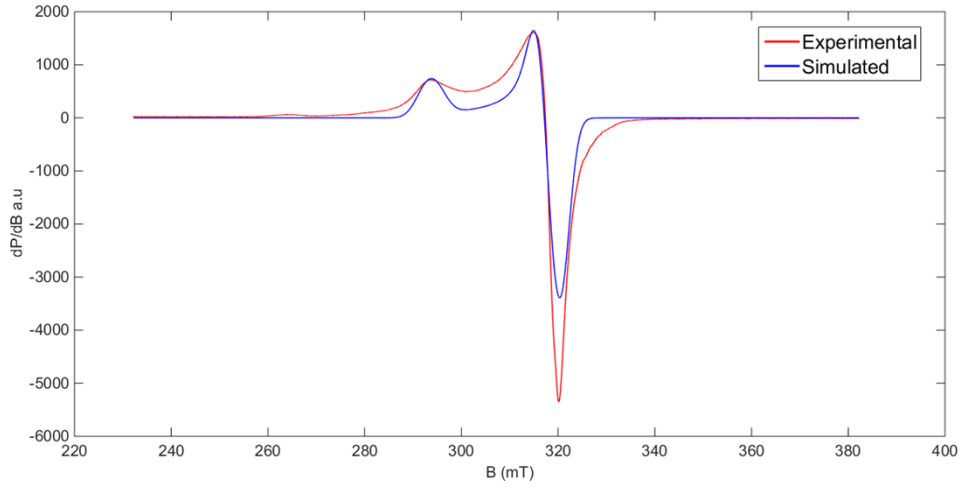


Fig. S9. The EPR spectrum of BMPC in their polycrystalline state at 298 K.

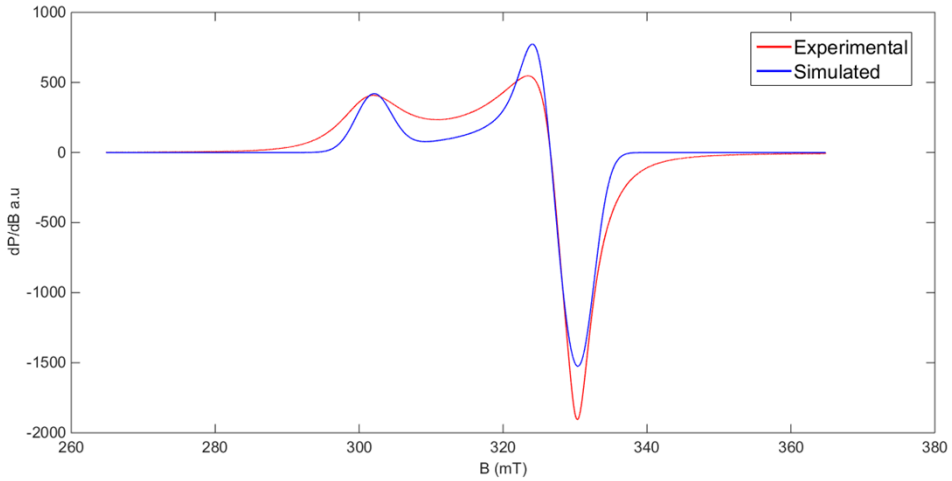


Fig. S10. The EPR spectrum of BMPC in DMF as solvent at 77 K.

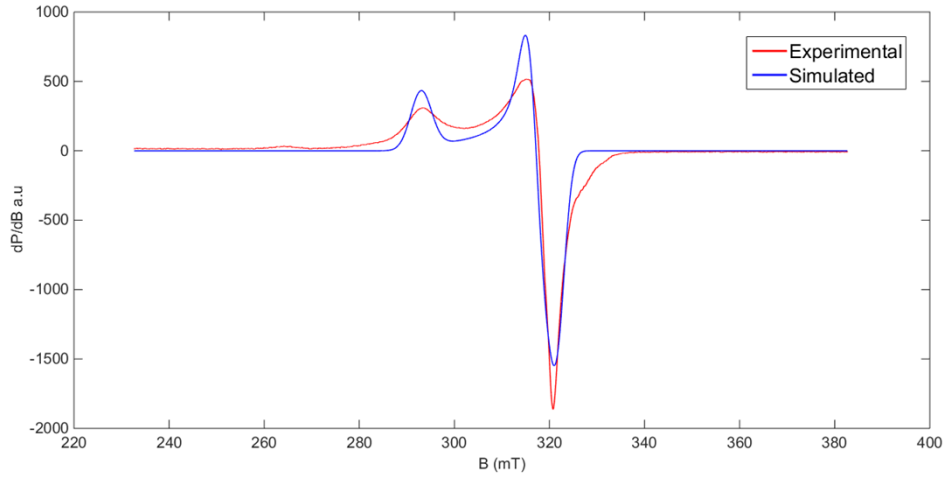


Fig. S11. The EPR spectrum of BNPC in their polycrystalline state at 298 K.

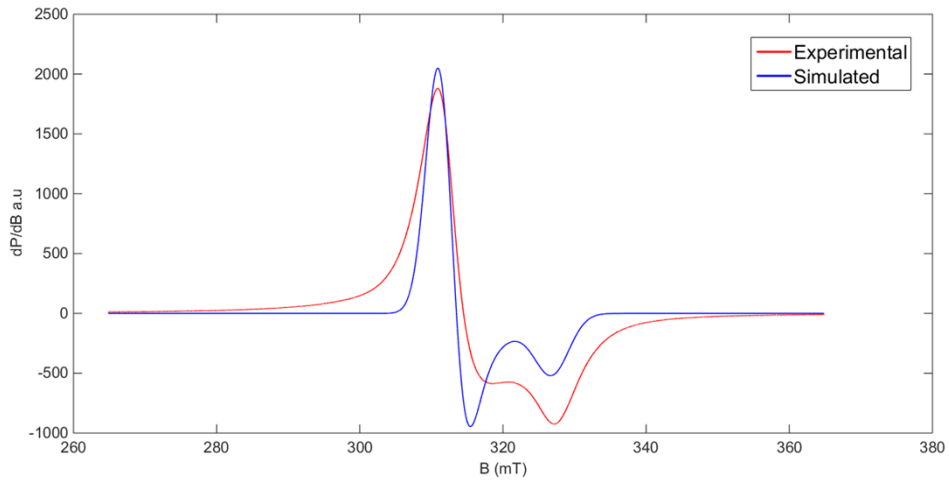


Fig. S12. The EPR spectrum of BNPC in DMF as solvent at 77 K.

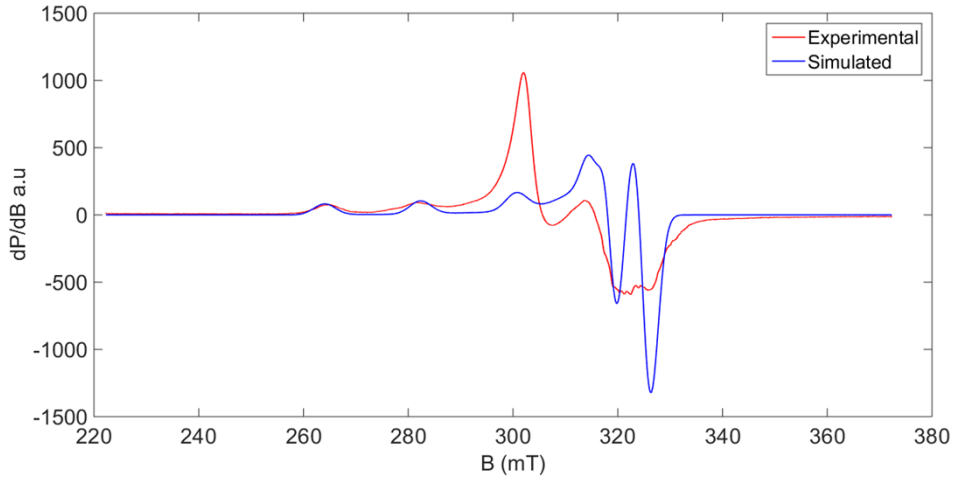


Fig. S13. The EPR spectrum of 4BNPC in their polycrystalline state at 298 K.

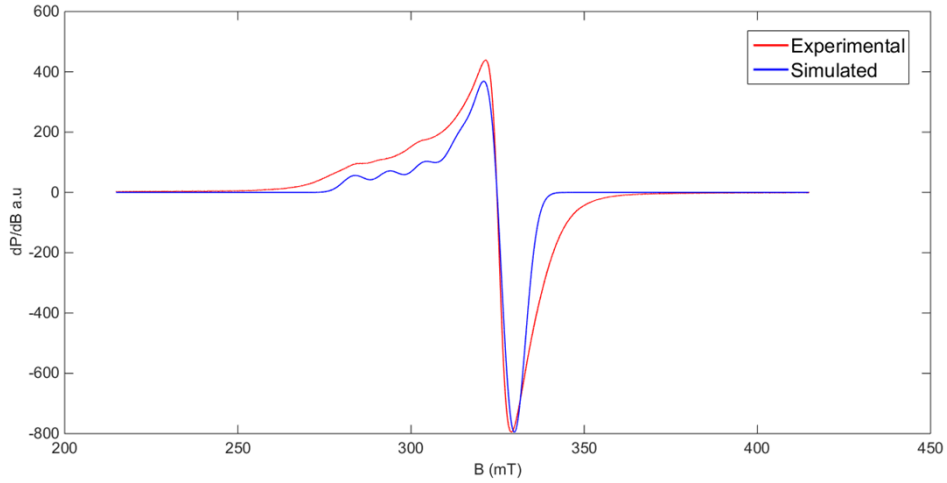


Fig. S14. The EPR spectrum of 4BNPC in DMF as solvent at 77 K.

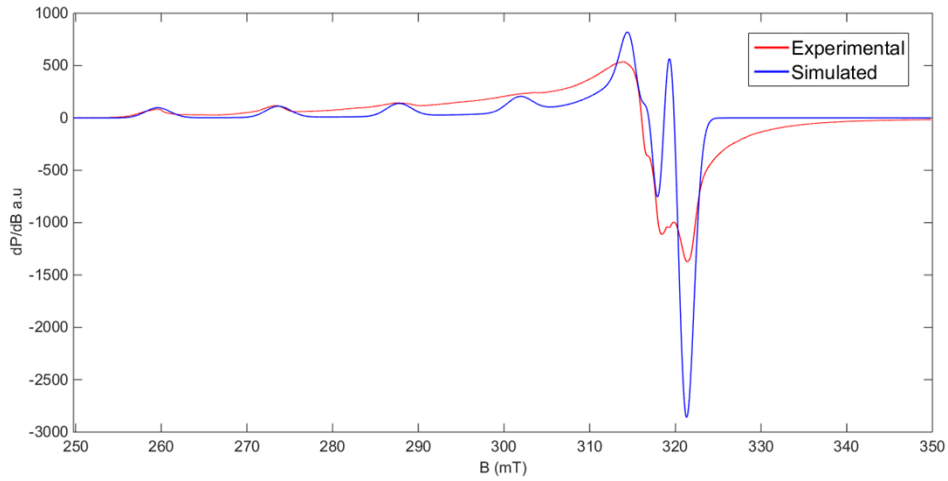
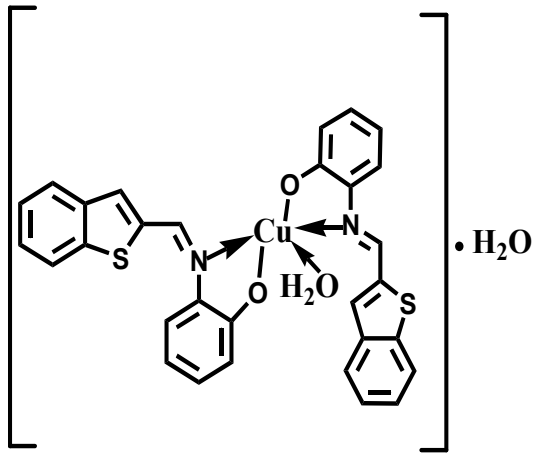
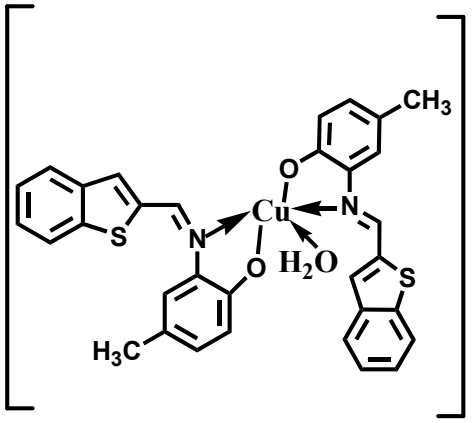


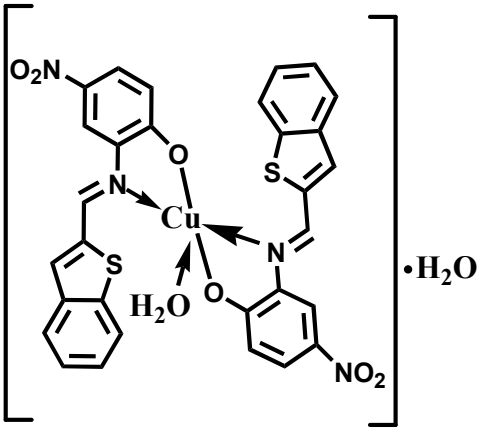
Fig. S15. Proposed structures of Cu(II) complexes.



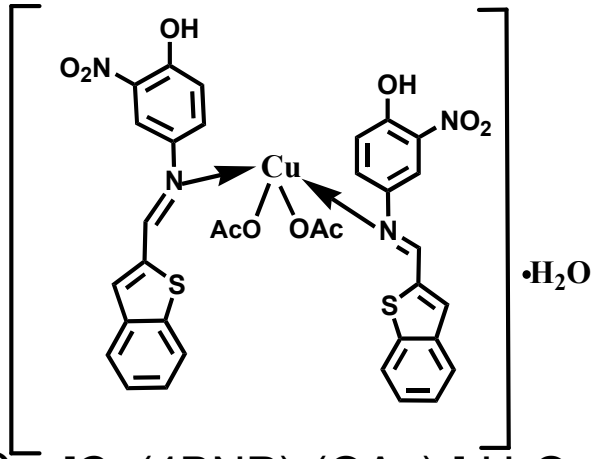
$[Cu(BA)_2(H_2O)].$



$[Cu(BMP)_2H$



$[Cu(BNP)_2(H_2O)].H_2O$



$[Cu(4BNP)_2(OAc)_2].H_2O$

Fig. S16. The MM plot showing the α-amylase inhibition of the synthesized Cu(II) complexes.

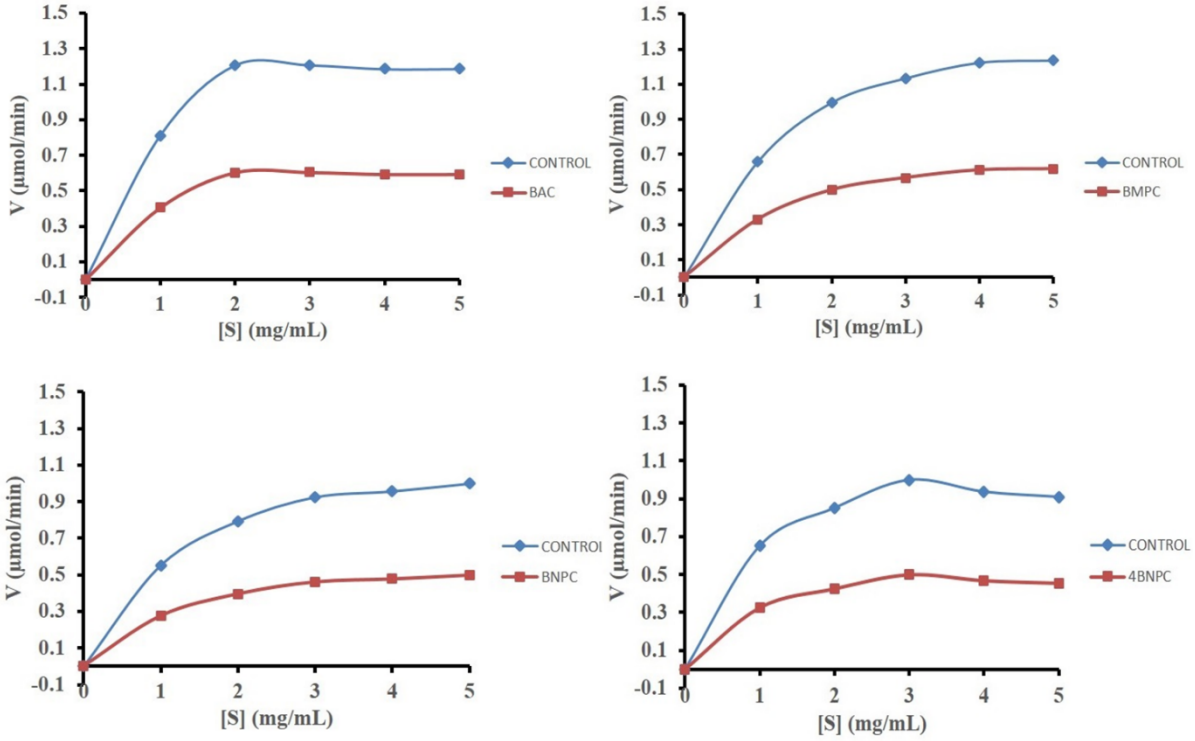


Fig. S17. The LB plot analysis showing the α-amylase inhibition of the synthesized Cu (II) complexes.

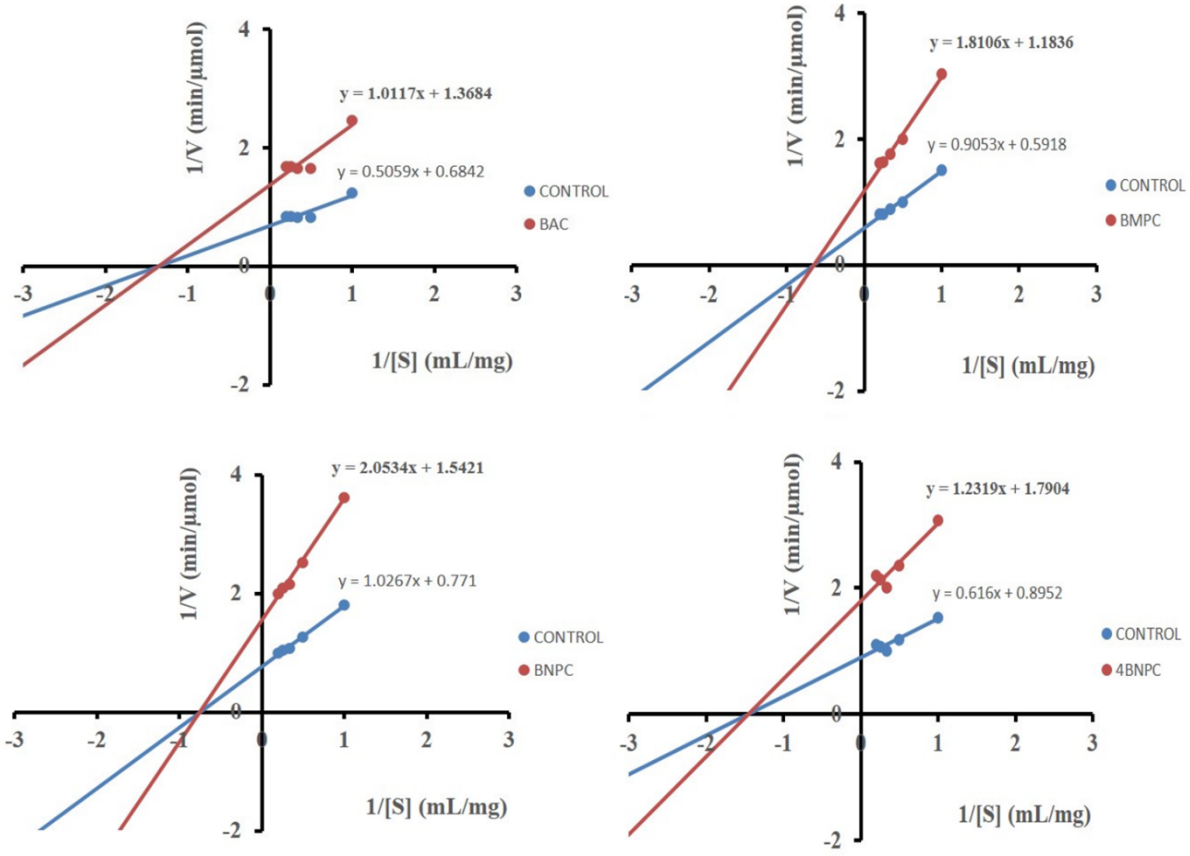


Table S1. The analytical data of the Cu(II) complexes derived from the Schiff base ligands.

Complex	Molecular Formula (M.F.)	Formula Weight	Color (Yield %)	Element Analysis Found (calc) %				% of Metal Found (calc)
				C	H	N	S	Cu
BAC $[Cu(BA)_2(H_2O)].H_2O$	$C_{30}H_{24}CuN_2O_4S_2$	603.06	Dark brown (78)	59.64 (59.68)	4.00 (4.04)	4.64 (4.68)	10.61 (10.65)	10.52 (10.56)
BMPC $[Cu(BMP)_2H_2O]$	$C_{32}H_{26}CuN_2O_3S_2$	613.08	Black (80)	62.57 (62.61)	4.27 (4.31)	4.56 (4.60)	10.44 (10.48)	10.35 (10.39)
BNPC $[Cu(BNP)_2(H_2O)].H_2O$	$C_{30}H_{22}CuN_4O_8S_2$	693.03	Dark brown (77)	51.90 (51.94)	3.19 (3.23)	8.07 (8.11)	9.24 (9.28)	9.15 (9.19)
4BNPC $[Cu(4BNP)_2(OA_C)_2].H_2O$	$C_{36}H_{34}CuN_4O_{11}S_2$	825.11	Dark brown (76)	52.32 (52.36)	4.15 (4.19)	6.78 (6.82)	7.76 (7.80)	7.69 (7.73)

Table S2. The molar conductivity and magnetic moment values of the Cu(II) complexes.

Complex	Molar Conductance ($Ohm^{-1}cm^2mol^{-1}$)	μ_{eff} (B.M.)
BAC $[Cu(BA)_2(H_2O)].H_2O$	8.12	1.88
BMPC $[Cu(BMP)_2H_2O]$	9.20	1.89
BNPC $[Cu(BNP)_2(H_2O)].H_2O$	12.23	1.84
4BNPC $[Cu(4BNP)_2(OA_C)_2].H_2O$	18.06	1.92

Table S3. The spectral assignments recorded for the Cu(II) complexes from the UV-Visible spectrum.

Metal Complex	UV-Vis Spectral Bands: nm (cm^{-1})	Log ε ($L\ mol^{-1}cm^{-1}$)	Assigments of the Spectral Bands
BAC $[Cu(BA)_2(H_2O)].H_2O$	304 (32890)	2.93	Intra ligand transition
	358 (27930)	2.82	Intra ligand transition
	380 (26310)	2.79	Intra ligand transition
	465 (21500)	2.19	$^2B_{1g} \rightarrow {}^2Eg\ (dx^2-y^2 \rightarrow dxz, dyz)$
	558 (17920)	1.86	$^2B_{1g} \rightarrow {}^2B_2g\ (dx^2-y^2 \rightarrow dxy)$
BMPC $[Cu(BMP)_2H_2O]$	319 (31340)	3.01	Intra ligand transition
	349 (28650)	2.93	Intra ligand transition
	384(26040)	2.22	Intra ligand transition
	480(20830)	1.99	$^2B_{1g} \rightarrow {}^2Eg\ (dx^2-y^2 \rightarrow dxz, dyz)$
	548(18240)	1.86	$^2B_{1g} \rightarrow {}^2B_2g\ (dx^2-y^2 \rightarrow dxy)$
BNPC $[Cu(BNP)_2(H_2O)].H_2O$	296 (33780)	3.19	Intra ligand transition
	412 (24270)	3.16	Intra ligand transition
	455 (21970)	3.19	Intra ligand transition
	472 (21180)	3.17	$^2B_{1g} \rightarrow {}^2Eg\ (dx^2-y^2 \rightarrow dxz, dyz)$
	605 (16520)	2.24	$^2B_{1g} \rightarrow {}^2B_2g\ (dx^2-y^2 \rightarrow dxy)$
4BNPC $[Cu(4BNP)_2(OAc)_2].H_2O$	282(35460)	2.97	Intra ligand transition
	343(29150)	2.74	Intra ligand transition
	427(23410)	2.63	Intra ligand transition
	495(20200)	2.31	$^2B_{1g} \rightarrow {}^2Eg\ (dx^2-y^2 \rightarrow dxz, dyz)$
	564(17730)	1.34	$^2B_{1g} \rightarrow {}^2B_2g\ (dx^2-y^2 \rightarrow dxy)$

Table S4. The characteristic stretching vibrational bands (cm^{-1}) observed for the Cu(II) complexes.

Compound	$\nu_{(OH)}$	$\nu_{(C=N)}$	$\nu_{(C-O)}$	$\nu_{(OAc)}$
BA	3424	1598	1294	-
BAC	3444	1570	1237	-
$[Cu(BA)_2(H_2O)].H_2O$				
BMP	3362	1599	1237	-
BMPC	3391	1578	1219	-
$[Cu(BMP)_2H_2O]$				
BNP	3354	1590	1274	-
BNPC	3406	1569	1180	-
$[Cu(BNP)_2(H_2O)].H_2O$				
4BNP	3422	1619	1236	-
4BNPC	3452	1579	1233	ν_{as} 1425
$[Cu(4BNP)_2(OA_C)_2].H_2O$				ν_s 1360

Table S5. The TG-DTG data recorded for the Cu(II) complexes derived from the Schiff base ligands BA, BMP, BNP and 4BNP.

Complexes	Decomposition Temperature (°C)	DTG Max (°C)	Estimated Weight Loss (%) Calc (Found)	Decomposition of Product Assignments
BAC	162-222	193	3.86 (3.97)	Loss of coordinated water
$[Cu(BA)_2(H_2O)].H_2O$	208-376	312	54.87 (53.06)	Organic moiety
	380-684	498	13.15 (14.77)	Organic moiety
BMPC	160-213	184	3.91 (4.13)	Loss of coordinated water
$[Cu(BMP)_2 H_2O]$	275-296	282	15.30 (16.38)	Elimination of one ligand molecule
	298-308	304	12.66 (13.15)	Organic moiety
	315-338	330	14.47 (15.89)	Organic moiety
BNPC	193-272	247	4.35 (4.73)	Loss of coordinated water
$[Cu(BNP)_2(H_2O)].H_2O$	268-340	307	12.22 (13.84)	Organic moiety
	367-461	397	20.30 (19.66)	Organic moiety
4BNPC	200-286	266	16.46 (15.79)	Loss of acetate moiety
$[Cu(4BNP)_2(OAc)_2].H_2O$	284-338	318	22.33 (24.05)	Organic moiety

Table S6. The values of the minimal inhibitory concentration of the chosen compound against bacterial strains.

Compounds	**MIC values (µg/mL)**			
	E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***
BAC	30.7	51.7	41.4	33.6
Ciprofloxacin	>7	0	>7	>7

Table S7. The percentage of the inhibition and the IC_{50} values of the synthesized Schiff base complexes of Cu(II).

Compound	**IC_{50} (mM) α-amylase**
Acarbose	0.033 ±0.008
BAC	0.040 ± 0.100
BMPC	0.085 ± 0.200
BNPC	-
4BNPC	0.100 ± 0.300

Table S8. The modulation of the α-amylase activity, impact on K_m, V_m, and K_i, inhibition mode on the Schiff base Cu(II) complexes.

Compound	**K_m (mM)**	**V_m (µmol/min)**	**K_i (mM)**	**Mode of Inhibition**
Control	0.739	1.462		
BAC	0.739	0.731	0.512	Non-Comp
Control	1.530	1.690		
BMPC	1.530	0.845	0.397	Non-Comp
Control	1.332	1.297		
BNPC	1.332	0.649	0.449	Non-Comp
Control	0.688	1.117		
4BNPC	0.688	0.559	0.378	Non-Comp

Table S9. The percentage of the cell viability of the Cu(II) complexes on the H9c2 cell line.

Compound	1 µg/mL	5 µg/mL	10 µg/mL	25 µg/mL
BAC	81.16	51.68	28.26	21.55
BMPC	82.69	66.05	32.55	-3.63
BNPC	65.20	46.89	28.66	-4.46
4BNPC	68.26	47.32	31.61	-6.98

Table S10. The percentage of the cell viability of the Cu(II) complexes on the HepG2 cell line.

Compound	1µg/mL	5 µg/mL	10 µg/mL	25 µg/mL
BAC	15.17	9.74	-11.43	-65.26
BMPC	-5.89	-16.45	-77.12	-100.00
BNPC	20.28	13.84	10.77	9.76
4BNPC	19.63	12.32	9.51	8.22

Table S11. The percentage of the cytotoxicity of the Schiff base complexes of Cu(II) on the DLA cell line.

Compound	10 µg/mL	20 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
BAC	22.87	22.87	28.91	33.66	41.44
BMPC	38.30	41.61	47.38	62.77	100
BNPC	32.66	32.66	32.66	33.83	35.97
4BNPC	11.99	24.48	24.48	35.33	40.67

Table S12. The global descriptors of the Schiff base ligands including the chemical potential, chemical hardness, chemical softness, electronegativity nucleophilicity, and electrophilicity are calculated using **Equations S1 to S6.**

The theorem developed by Koopman's, 'I' denotes the ionization potential obtained from -$E_{HOMO,}$ and 'A' is the electron affinity equal to $-E_{LUMO}$.

$$\text{Electronegativity } (\chi) = \frac{I + A}{2} \qquad \text{Eqn. (S1)}$$

$$\text{Chemical hardness } (\eta) = \frac{I - A}{2} \qquad \text{Eqn. (S2)}$$

$$\text{Chemical potential } (\mu) = -\chi \qquad \text{Eqn. (S3)}$$

$$\text{Chemical softness } (S) = \frac{1}{2\eta} \qquad \text{Eqn. (S4)}$$

$$\text{Electrophilicity index } (\omega) = \frac{\mu\text{^}2}{2\eta} \qquad \text{Eqn. (S5)}$$

$$\text{Nucleophilicity index } (n) = 1/\omega \qquad \text{Eqn. (S6)}$$

Table S13. Quantum descriptors for the complex BAC

Quantum descriptors	eV
HOMO	-5.23
LUMO	-2.60
Energy Gap ΔE	2.66
Ionization Energy (I = ε HOMO = -HOMO)	5.23
Electron Affinity (A= ε LUMO = -LUMO)	2.60
Global Hardness (η = (I-A)/2)	1.31
Global Softness (S = 1/η)	0.76
Chemical Potential (μ =- (I+A)/2)	-3.92
Electronegativity (χ = -μ)	3.92
Electrophilicity index (ω = μ2/2η	5.84
Nucleophilicity index (N = 1/ω)	0.17
ΔN max	2.98
Electron accepting power ω+= A2/2(I-A)	1.29
Electron donating power ω+= I2/2(I-A)	10.60

Table S14. Quantum descriptors for the complex BMPC

Quantum descriptors	eV
HOMO	-4.95
LUMO	-2.55
Energy Gap ΔE	2.44
Ionization Energy (I = ε HOMO = -HOMO)	4.95
Electron Affinity (A= ε LUMO = -LUMO)	2.55
Global Hardness (η = (I-A)/2)	1.20
Global Softness (S = 1/η)	0.83
Chemical Potential (μ =- (I+A)/2)	-3.75
Electronegativity (χ = -μ)	3.75
Electrophilicity index (ω = μ2/2η	5.84
Nucleophilicity index (N = 1/ω)	0.17
ΔN max	3.12
Electron accepting power ω+= A2/2(I-A)	1.35
Electron donating power ω+= I2/2(I-A)	9.11

Table S15. Quantum descriptors for the complex BNPC

Quantum descriptors	eV
HOMO	-6.18
LUMO	-3.24
Energy Gap ΔE	2.98
Ionization Energy (I = ε HOMO = -HOMO)	6.18
Electron Affinity (A= ε LUMO = -LUMO)	3.24
Global Hardness (η = (I-A)/2)	1.47
Global Softness (S = 1/η)	0.68
Chemical Potential (μ =- (I+A)/2)	-4.71
Electronegativity (χ = -μ)	4.71
Electrophilicity index (ω = μ2/2η	7.53
Nucleophilicity index (N = 1/ω)	0.13
ΔN max	3.20
Electron accepting power ω+= A2/2(I-A)	1.78
Electron donating power ω+= I2/2(I-A)	10.80

Table S16. Quantum descriptors for the complex 4BNPC

Quantum descriptors	eV
HOMO	-6.25
LUMO	-2.98
Energy Gap ΔE	3.35
Ionization Energy (I = ε HOMO = -HOMO)	6.25
Electron Affinity (A= ε LUMO = -LUMO)	2.89
Global Hardness (η = (I-A)/2)	1.68
Global Softness (S = 1/η)	0.60
Chemical Potential (μ =- (I+A)/2)	-4.57
Electronegativity (χ = -μ)	4.57
Electrophilicity index (ω = μ2/2η	6.23
Nucleophilicity index (N = 1/ω)	0.16
ΔN max	2.72
Electron accepting power ω+= A2/2(I-A)	1.25
Electron donating power ω+= I2/2(I-A)	16.06

Among these complexes 4BNPC showed high global hardness and low softness value so we can predict the following properties such as high stability, low reactivity, strong bonds, potential catalytic activity, biological interactions, optical and electronic properties.