

ANTIMICROBIAL ACTIVITY AND COMPUTATIONAL STUDY OF NEW COBALT (II) COMPLEX OF BENZOTHAZOLE DERIVATIVE

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ABSTRACT

Condensation of 6-methoxy benzothiazol-2-amine with 2-hydroxy-naphthaldehyde gave new Schiff-base derivative in good yield. The metal complex of Co(II) have been synthesized with Schiff base of benzothiazol derivative. Spectroscopic study such as, two dimensional NMR of new compound have been obtained by using nuclear magnetic resonance 600 MHz.

Computational study of all compounds was calculated using Gaussian 09 program package. The synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*. Additionally, the compounds were tested for antifungal activity against *Candida albicans*, *Candida tropicalis*, *Aspergillus multi* and *Aspergillus niger*. Cobalt complex compound exhibited more activity as antifungal than benzothiazole derivative.

INTRODUCTION

The condensation products of primary amines with carbonyl compounds were first reported by Schiff in 1864 and the products are often referred to as Schiff bases⁽¹⁻³⁾. Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical and biological activities⁽⁴⁾ With the increasing incidence of deep mycosis, there has been increasing emphasis on the screening of new and more effective antimicrobial drugs with

low toxicity⁽⁵⁾. Benzothiazole is a privileged bicyclic ring system. Due to its potent and significant biological activities it has great pharmaceutical importance; hence, synthesis of this compound is of considerable interest. The small and simple benzothiazole nucleus if present in compounds involved in research aimed at evaluating new products that possess interesting biological activities. 2-substituted benzothiazole has emerged in its usage as a core structure in the diversified therapeutically applications. The studies of structure–activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity⁽⁶⁾. In 1887, substituted benzothiazole was first synthesized by A. W. Hofmann then because of diversified activity as well as simple cyclization mechanism number of synthetic routes have been adopted and reported⁽⁶⁾.

Benzothiazole derivatives were reported for their diversified activity viz., antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antifungal, a topical carbonic anhydrase inhibitor and an antihypoxic⁽⁷⁾. The aim of the present work was to synthesis some new complex of bezothiazole derivatives, in the hope to use them as antimicrobial compounds.

MATERIALS AND METHODS

a) Physical measurmenets

One dimensional ^1H , ^{13}C NMR and two dimensional COSY, HSQC and HMBC NMR spectra were measured on a Bruker at 600 MHz, with TMS as internal reference at Konstanz university, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

b) Synthesis

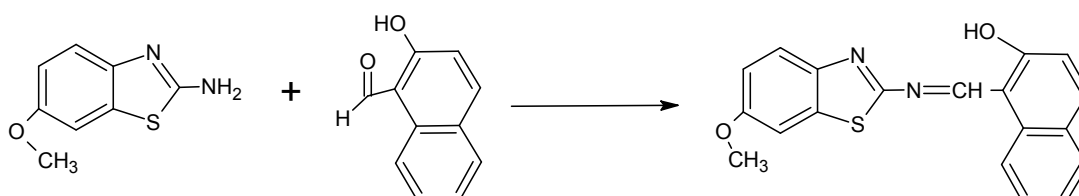
Synthesis of Schiff- base

2.77 mmol (0.5g) of 6-methoxy-1, 3- benzothiazol-2-amine in 25 ml ethanol was added to 2.77 mmol (0.477g) of hot ethanolic solution of 2-hydroxy naphthaldehyde, two drops of glacial acetic acid was added and resulting solution was refluxed for 3h and then left over night in refrigerator, the solid product obtained was filtered and washed with acetone and the final product was recrystallized by using chloroform: ethanol 8:2 to yield orange crystals of 1-[(6-methoxy-1,3- benzothiazol-2-yl) carbonimidoyl]naphthalen-2-ol.

Yield;

80% , M.P.= 192-194⁰C. FT-IR(KBr, Cm^{-1}), 3475(O-H), 2894(C-H), 2830(C-H),

1622(C=N), 1602(C=C), 1190(C-O); ^1H NMR(DMSO- d_6); δ 3.66(s,3H-OCH $_3$); 7.12-8.77(m,9H-Ar-H); 9.98(s,1H,CH=N); 13.78(s,1H-OH). ^{13}C NMR(DMSO- d_6); 56.25(OCH $_3$), 105.88-146.95(C-Ar), 157.8(C-OH), 162.8(C-CH=N), 164.8(C-C=N), 167.1(C-C-S); *Anal.* for C $_{19}$ H $_{14}$ N $_2$ O $_2$ S (M.wt 334):Calc. C, 68.26; H, 4.19; N, 8.38; Found: C, 68.45; H, 4.62; N, 8.53.



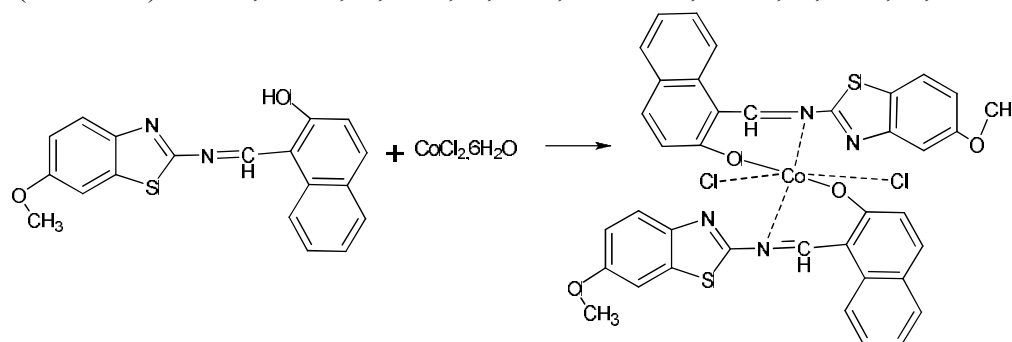
Scheme 1; Synthesis of Schiff-base of benzothiazol derivative (Ligand)

Synthesis of Cobalt complex

For the synthesis of complexes (0.5g,1.5 mmol) Schiff-base of 6-methoxy benzothiazole (L) solution was prepared in 60% ethanol–water solvent and refluxed for three hours with (0.179 g, 0.75 mmol) solution of cobalt chloride hexa hydrate. The refluxed solutions were kept for one day. Solid crystalline compounds appeared in the solution, which were filtered, washed with 60% acetone–water mixture, dried and weighed. Melting point of the complexes was recorded.

Yield;

73% , M.P.= 202-204 $^{\circ}$ C. IR(KBr, Cm $^{-1}$),2950(C-H), 2890(C-H), 1608(C=N), 1598(C=C), 1196(C-O); ^1H NMR(DMSO- d_6) δ 3.73(s,6H-OCH $_3$);7.24-8.94(m,18H-Ar-H); 10.82(s,2H,CH=N);12.02(s,2H-OH); ^{13}C NMR(DMSO- d_6); 56.25(OCH $_3$), 100.01-145.95(C-Ar), 157.81(C-OH), 162.90(C-CH=N), 164.87(C-C=N), 167.22(C-C-S); *Anal.* for C $_{38}$ H $_{26}$ N $_4$ O $_4$ S $_2$ CoCl $_2$ (M.wt 796): (M.wt 796):Calc.C, 57.28; H, 3.26; N, 7.03; Found: C, 57.45; H, 3.67; N, 7.33.



Scheme 2; Synthesis of Cobalt complex of benzothiazol derivative (Complex)

Computational study

The quantum chemical calculations were performed by density functional theory (DFT) with Gaussian 09 package. The geometry of the ligand and complex were optimized at the hybrid functional Becke's three parameter and the Lee, Yang, Parr (B3LYP) as a level of theory and 6-311G as a basis set.

Antimicrobial activity

The synthesized compounds were screened *in vitro* for their antibacterial activity against: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*. Additionally, the compounds were tested for antifungal activity against *Candida albicans*, *Candida tropicalis*, *Aspergillus multi* and *Aspergillus niger*, using the paper disc-agar diffusion technique on Muller Hinton agar as a culture media for antibacterial activity⁽⁸⁾. The test compounds were dissolved in DMSO solvent and recommended concentrations (50, 100 and 200µg/mL) were used in the disc-agar diffusion technique. Antibiotic drug Ampicillin and Nystatin were used as control for bacteria and fungi, respectively. Petri plates containing 20 mL of Mueller Hinton Agar were used for all the bacteria tested. *Aspergillus niger*, *Aspergillus multi* and *Candida spp.* strains were cultivated in Sabouraud dextrose agar. Sterile Whatman no. 1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test were placed on the Petri plates. A paper disk impregnated with dimethylsulfoxide (DMSO) was used as negative control. The plates were incubated for 24 h at 37°C in the case of bacteria and 72 h at 27°C for fungi. The inhibition zone diameters were measured in millimeters.

RESULTS AND DISCUSSION

Schiff bases are versatile intermediates in organic synthesis and have been used to prepare numerous pharmacologically important compounds⁽⁹⁾. In the present work a new ligand (L) derived from benzothiazole and cobalt complex compounds were prepared by convenient method. The preparation of azomethine compound based on condensation of 6-methoxy benzothiazole with 2-hydroxy naphthaldehyde using glacial acetic acid. The new compound was obtained as orange solid in 80% yield. Complexation of Schiff-base with cobalt chloride gave a new cobalt complex compound as yellow crystal in good yield (Experimental section).

The new compounds are stable toward moisture and air. They were characterized by NMR (one and two dimensional) and elemental analysis (CHN). IR spectra for synthesized compounds displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The IR spectra of Schiff base(L) show broad strong bands in 3475 cm⁻¹ due to ν(O-H) which this band was disappeared in complex compound. The IR spectra confirm the presence of the imine groups (-CH=N-) stretching with a sharp region around 1622 -1608 cm⁻¹. The IR spectra of two compounds show a band at 1602-1598 cm⁻¹ range can be attributed to C=C aromatic. The azomethine vibration of the Schiff base ligand was appeared at 1622 cm⁻¹. Because of bond formation between the metal and the imine nitrogen, the C=N bond stretching

was shifted to lower frequencies relative to the free Schiff base and appeared in 1608 cm^{-1} for the metal complex⁽¹⁰⁾.

^1H NMR spectra of Schiff base (L) were recorded in DMSO-d_6 solution and show all the expected protons with proper intensity ratio, Fig.1. In two compounds the signal for protons of methoxy groups appear as singlet in the region 3.66-3.73 ppm, The aryl protons show the expected resonance for substituted benzene. The proton of azomethine resonate as a singlet between 9.98-10.82 ppm for ligand and complex compounds respectively. The OH group in both compounds appear as singlet in the region 12.02 and 13.78 ppm, Fig.1. The ^{13}C NMR spectra of ligand and complex compounds show the expected resonance signals and is consistent with their structure. The large variation of carbon atoms bearing sulphur can be explained by the polarity of the C-S bond in thiazole ring, Fig.1.

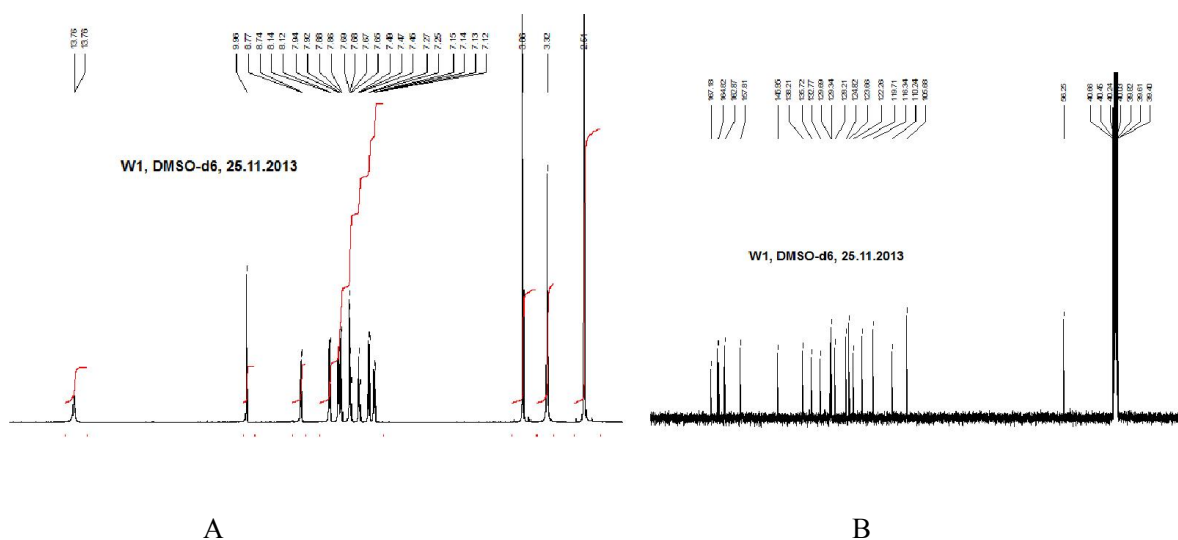


Fig.1: A) ^1H NMR, B) ^{13}C NMR of benzothiazole derivative (L).

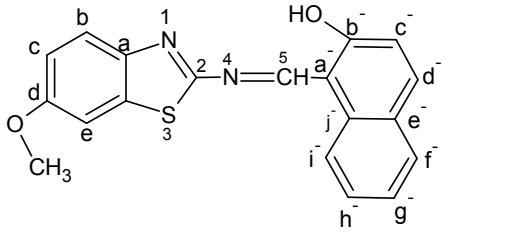
NMR Study

a- 2D COSY NMR Spectra

The 2D COSY NMR (600 MHz) of Schiff base of benzothiazole (L) shows the coupling of each two protons. The cross-peak show protons signals a 7.87 and 7.47 ppm due to coupling of (H-h^- and H-g^-) and coupling at 7.88 and 7.25 ppm due to (H-c^- and H-d^-), the two peaks at 8.13

and 7.93 ppm due to (H-c and H-b), and the peaks at 7.47 and 7.14 ppm due to (H-f and H-g⁻), Table 1, Fig.2.

Table (1): COSY data for Schiff-base of 6-methoxy benzothiazole (L)

Compound	¹ H (ppm)	¹ H (ppm)	Assignment
	7.87	7.47	h ⁻ - g ⁻
	7.88	7.25	c ⁻ - d ⁻
	8.13	7.93	c - b
	7.47	7.14	f - g ⁻
	7.87	7.12	h ⁻ - i ⁻

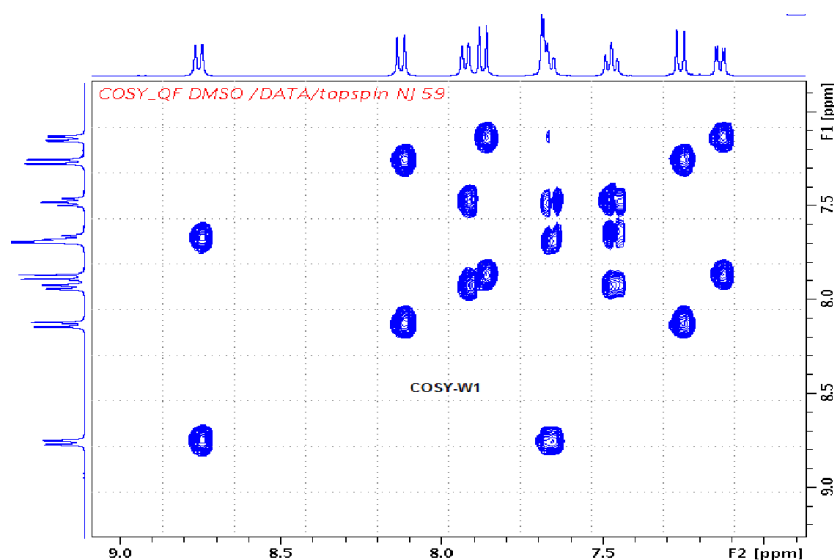
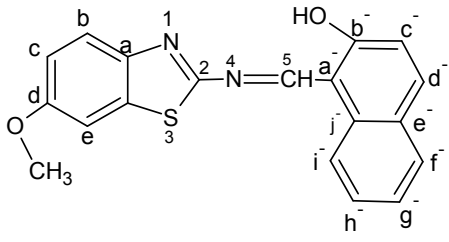


Fig. 2. COSY NMR spectra of Schiff-base of 6-methoxy benzothiazole (L).

b- HSQC NMR Spectra

The HSQC NMR spectrum of (L) shown that the ¹³C peak at 56.2 ppm is bonded to the ¹H with peak at 3.8 ppm. Thus, the correlation of protons and carbon in aromatic rings such as in positions (e and c), (c and d), (i⁻ and h), (g⁻ and e⁻) and in positions b and d are shown in Table 2, Fig. 3.

Table (2) HSQC data for Schiff-base of 6-methoxy benzothiazole (L)

Compound	^1H (ppm)	^{13}C (ppm)	Assignment
	3.86	56.25	C,H (OCH ₃)
	7.85	129.9	C,H (e, c)
	8.13	138.21	C,H (c, d)
	7.93	132.7	C,H (b)
	7.12	116.24	C,H (i, h)
	7.25	120.0	C,H (d)
	7.47	124.0	C,H (g, e)

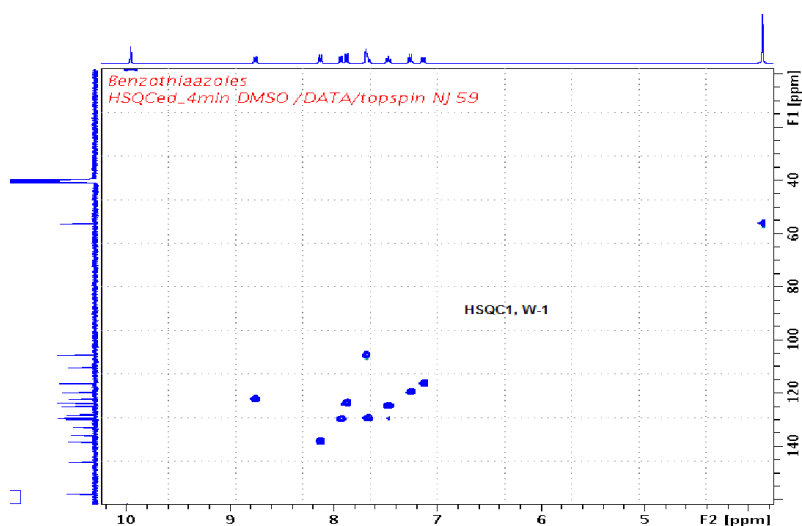


Fig. 3. HSQC NMR spectra of Schiff-base of 6-methoxy benzothiazole (L).

c- HMBC NMR Spectra

The HMBC spectrum is used to correlate, or connect, ^1H and ^{13}C peaks for atoms separated by multiple bonds (usually 2 or 3)⁽¹¹⁾.

The spectrum (600MHz) of (L) compound (Fig.4) shows that in aromatic rings, the most common correlations is seen in region 7.47-8.13 ppm, the correlation of protons and multi carbons are, H(e) and C(a,b,e), H(e) and C(f,e), H(g) and C(g, f), H(b) and (c, b, d), H(h) and (f, e). The COSY and HSQC NMR of cobalt complex compound are shown in Figs, 5 and 6.

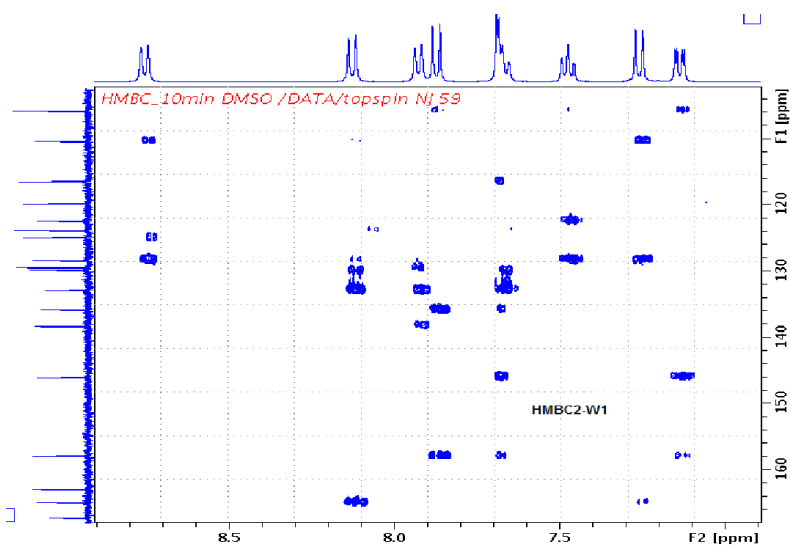


Fig. 4. HMBC NMR spectra of Schiff-base of 6-methoxy benzothiazole (L).

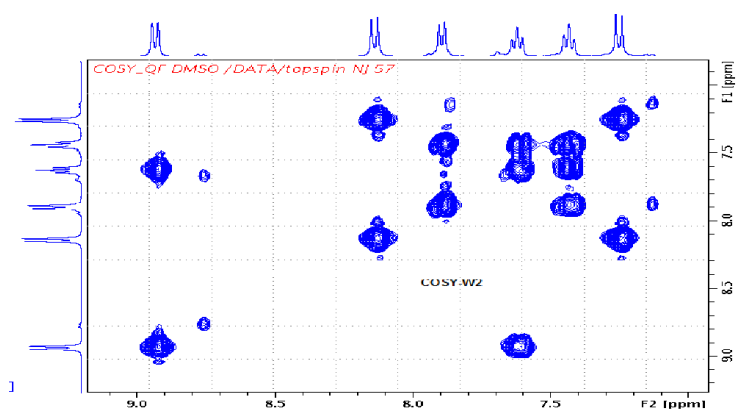


Fig. 5. COSY NMR spectra of cobalt complex compound

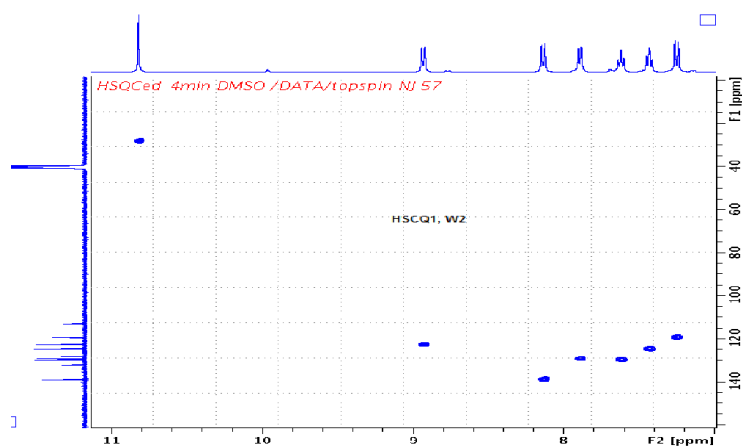


Fig. 6. HSQC NMR spectra of cobalt complex compound.

Computational study

The B3LYP has long been recognized as a good tool due to the fact that it is computationally less demanding for inclusion of electron correlation and could provide accurate geometries⁽¹²⁾ meanwhile, a previous study⁽¹³⁾.

The optimized structure of the ligand and Co(II) complex with labeling of the atoms are presented in Figs. 7 and 8 respectively. The optimized structure parameters, bond lengths, bond angles and dihedral angles are listed in Table 3 .

The ligand molecule deprotonates and then acts as a bidentate monoanionic Schiff-base ligand framework, which has a N, O⁻ binding mode. The calculated bond length C₂₂-N₂₁ in ligand is 1.323 Å which is the approximate value for a C=N double bond length, this a bond is elongation in the complex molecules to 1.352 Å because the coordination between lone pair on the nitrogen atom and d-orbital of cobalt atom. This case observed in IR spectrum through the shifting of stretching vibration position of C=N bond from 1622 cm⁻¹ to 1608 cm⁻¹. The C₄-O₂₃ in ligand has single bond properties, the distance is 1.415 Å .While this bond in metal complex molecule has the single and double bond properties, the distance of C₁₂-O₁₅ and C₂₀-O₃₉ are 1.322 Å and 1.326 Å, respectively. These values suggest that electron density may be delocalized throughout the aromatic ring.

The calculated dihedral angles in ligand are N₂₁-C₁₇-S₁₈-C₁₁ (179°) and C₅-C₂₂=N₂₁-C₁₇ (175.5°), indicate the ligand molecule is slightly planner structure. Inversely the Co(II) complex molecule has nonplaner structure, Table(3). On the other hand the theoretical angle values for the Co(II) complex show some deviation from the octahedral geometry. For example, the calculated angles obtained O₁₅-Co-O₃₉ , N₃₇-Co-Cl₅₀ and N₃₇-Co-N₄₂ are 176.34°, 174.86° and 95.86°, respectively.

The atomic charges of the ligand and Co-complex calculated by the Mulliken method⁽¹⁴⁾ are given in Table(4). As can be seen, high charge electrophalic density was found at oxygen and nitrogen atoms and charge of the N₂₁ atom in the ligand (which is -0.364) is higher than charge of N₁₆ atom(-0.253). Hence, the N₂₁ atom has a higher ability for coordination of the metal ion than N₁₆ because its has more basedity⁽¹⁵⁾. On the other hand, the charges electrophalic density are increasing for the Co-complex compare with ligand, this case in agreement with a high biological activity of the complex, Table(6).

The Total energy, binding energy and HOMO-LUMO energy gap computed by using same method and basis set and summarized in Table(5). The highest occupied molecular orbital

(HOMO) and the lowest unoccupied molecular orbital (LUMO) and their energy gap reflect the chemical activity of the molecule⁽¹⁶⁾. From values of HOMO-LUMO energy gap observed the Co-complex molecule has higher reactivity than ligand molecule ($\Delta E_{\text{LUMO-HOMO complex}} = 1.527\text{eV}$, ligand $= 1.908\text{eV}$) which is good in agreement with the results that obtained from biological activity tests. The HOMO and LUMO orbitals are depicted in Figure(9). The HOMO orbitals are localized mainly on phenyl rings, Nitrogen atoms, Oxygen atoms and Cobalt atom moieties. Whereas the LUMO of π nature are mostly located on the phenyl ring.

Table 3. Selected bond lengths , bond angles and dihedral angles of ligand and complex calculated with B3LYP/6-311G method.

Bond length(Å)	C ₁₇ -N ₂₁	C ₂₂ =N ₂₁	C ₁₇ -S	C ₅ -C ₂₂	C ₄ -O ₂₃	O ₂₃ -H ₂₄	Co-O	Co-N	Co-Cl
Ligand	1.380	1.323	1.902	1.458	1.415	0.991	-	-	-
Complex	1.410	1.352	1.953	1.419	1.326	-	1.924	2.037	2.362
	1.396	1.352	1.885	1.417	1.322	-	1.917	2.030	2.348
Angles bond(°)	N-C-S	C=N-C	N-Co-N	Cl-Co-Cl	O-Co-O	N-Co-Cl			
Ligand	116.58	120.36	-	-	-	-			
Complex	121.25	115.09	95.86	87.20	176.35	174.86			
	120.90	119.87				173.71			
Dihedral angles(°)	N-C-S-C	C-C=N-C	N-Co-N=C	O-Co-N=C					
Ligand	179.14	175.57	-	-					
Complex	175.30	147.75	127.7	29.64					
	-179.50	171.92	-66.47	34.27					

Table 4. The Mulliken atomic charges of ligand and complex calculated with B3LYP/6-311G method.

Ligand	O ₂₃	N ₁₆	N ₂₁	S	O ₁₉	C ₄	C ₁₄	C ₁₇	C ₂₂	C ₅		
	-0.546	-0.253	-0.364	0.481	-0.475	-0.15	-0.15	-0.176	-0.046	0.029		
Complex	O ₁₅	N ₄	N ₃₇	S ₀	O ₃₅	C ₁₂	C ₃₀	C ₂	C ₃₈	C ₁₁	Cl ₃₉	Co
	O ₃₉	N ₃₂	N ₄₂	S ₃₄	O ₄₇	C ₂₀	C ₄₆	C ₃₃	C ₄₁	C ₂₁	Cl ₅₀	
	-0.406	-0.325	-0.196	-0.498	-0.581	-0.12	-0.098	-0.190	-0.066	0.022	-	0.687
	-0.405	-0.407	-0.195	-0.402	-0.583	-0.11	-0.099	-0.191	-0.068	0.023	-	0.463

Table 5. Values of total energy, binding energy and HOMO-LUMO energy gap of ligand and complex calculated with B3LYP/6-311G method.

	Total energy(eV)	Binding energy(eV)	HOMO energy (eV)	LUMO nergy(eV)	$\Delta E_{\text{LUMO-HOMO}}$
ligand	-1390.2820	-6.8659	-4.801	-2.893	1.908
Complex	-3845.88447	-13.9141	-4.801	-3.274	1.527

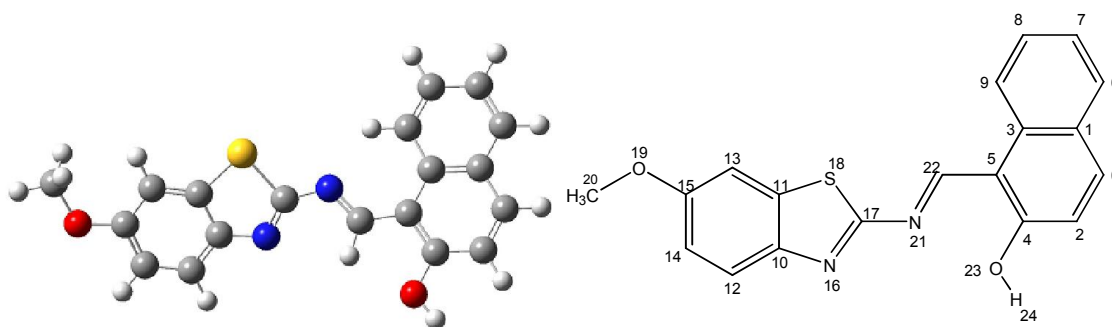


Figure (7): Optimized structure for Ligand together with its labeling

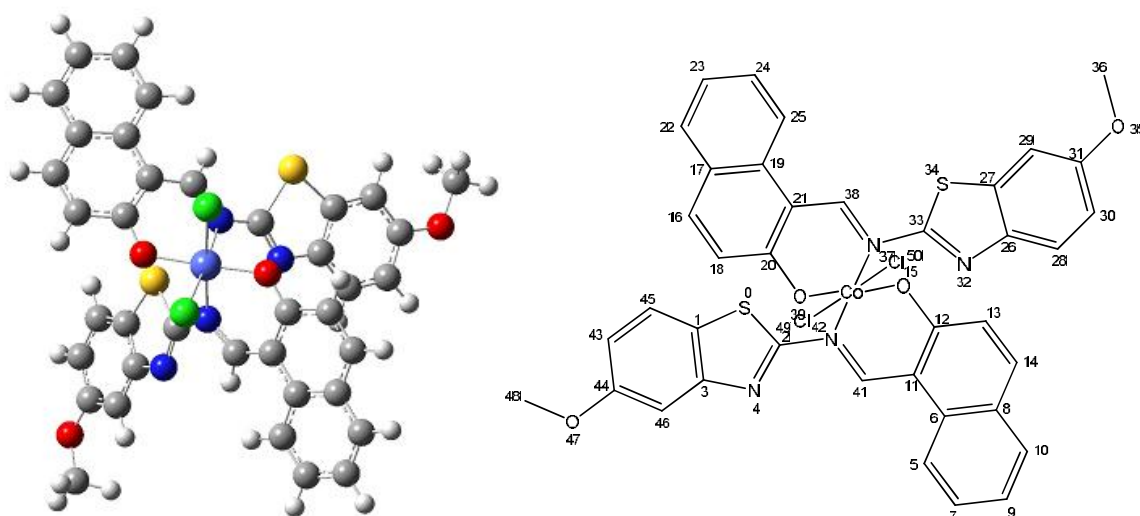


Figure (8): Optimized structure for Co complex together with its labeling

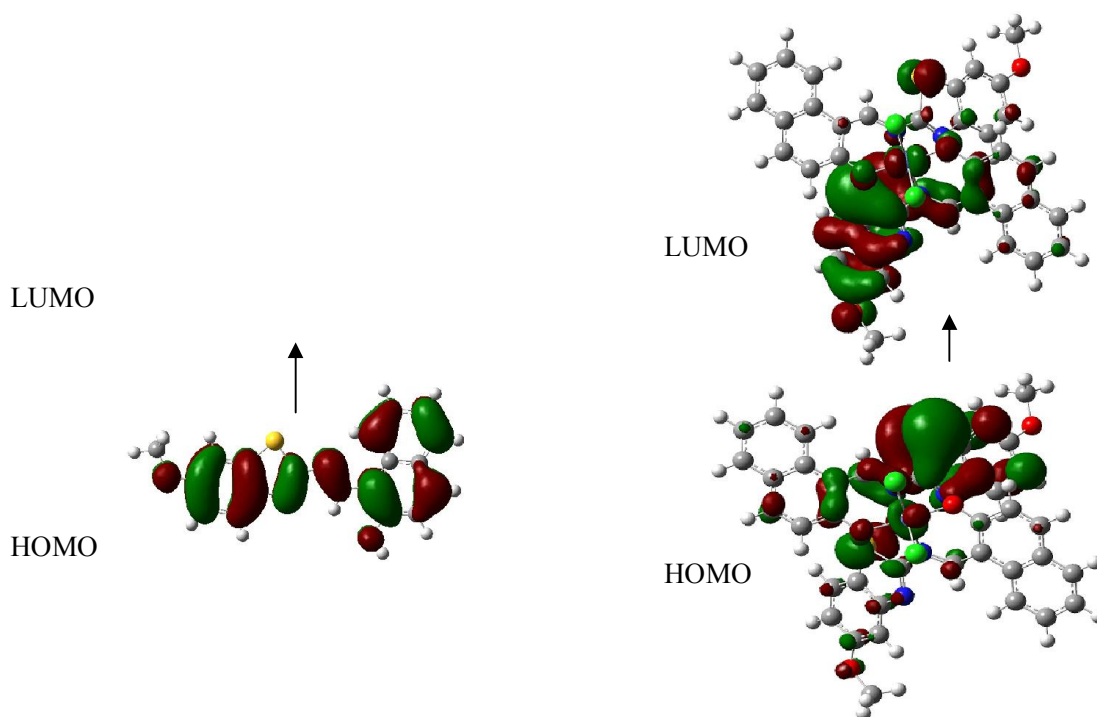


Figure (9): Representation of the HOMO and LUMO orbitals of ligand and complex.

Antimicrobial activity

The studied compounds have been screened for their *in vitro* antibacterial and antifungal activities, using the paper disc-agar diffusion technique⁽⁸⁾ by measuring the inhibition zone in mm. Antibiotic drug ampicillin and Nystatin were used as control for bacteria and fungi, respectively. The antibacterial activity of the synthesized compounds were tested against two

gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and two gram negative bacteria (*Klebsiella pneumonia*, *Escherichia coli*) at a concentration of 50, 100 and 200µg/mL using DMSO as a solvent, which not effected the growth of microbes. Mueller Hinton agar was used as culture media for antibacterial activity. The results of the antimicrobial activity are shown in Table (6).

It is observed that the activity of compounds increases with an increase in the concentration of the solutions. The synthesized compounds show activity against all the fungi species. However, the compounds had the highest effect against *Staphylococcus aureus*, but were inactive against *Klebsiella pneumonia* and active in high concentration against *Bacillus subtilis* and *Escherichia coli*.

The antifungal activity show more activity of cobalt complex compound than benzothiazole derivative.

Therefore, the possible explanations of our results attribute to the chemical structure of the bacterial cells wall which provides important ligands for adherence and receptor sites for antibiotics and drugs.

Table 6. Antibacterial activity of the studied compounds

Bacteria and fungi types	Antibiotic disc (control) (mm)		Zone inhibition of antimicrobial sensitivity test of compounds (mm)					
			Ligand (1)			Complex(2)		
			200	100	50	200	100	50
	Ampicillin	Nystatine						
1- <i>Klebsiella pneumonia</i>	40	-	0	0	0	0	0	0
2- <i>Escherichia coli</i>	20	-	15	0	0	0	0	0
3- <i>Staphylococcus aureus</i>	45	-	15	13	10	17	11	10
4- <i>Bacillus subtilis</i>	30	-	18	0	0	0	0	0
5- <i>Aspergillus niger</i>	-	15	20	15	15	25	22	20
6- <i>Aspergillus multi</i>	-	13	25	22	20	28	27	25
7- <i>Candida albicans</i>	-	12	18	15	12	16	15	12
8- <i>Candida tropica</i>	-	9	23	19	19	24	23	20

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الفعالية المايكروبية والدراسة الحاسوبية لمعقد الكوبلت لمشتق البنزو ثايازول الجديد

وصفي عبود المسعودي ، رشا منذر عثمان ، رافد حميدان الاسدي ، مهند أدريس علي

الخلاصة

تكثيف المركب 6-ميثوكسي بنزو ثايازول -2- أمين مع 2-هيدروكسي نافثالديهايد أعطت مشتق قاعدة شيف الجديدة بحصيلة انتاجية جيدة. حضر معقد الكوبلت بتفاعل الكوبلت الثنائي مع مشتق قاعدة شيف للثايازول. شخضت المركبات المحضرة ودرست طيفيا بأستخدام طيف الرنين النووي المغناطيس احادي وثنائي المحور 600 ميكايرتز . درست المركبات المحضرة حاسوبيا بأستخدام برنامج كاوسين 09
أختبرت المركبات المحضرة كمضادات بكتيرية ضد :

Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia.

اضافة الى اختبارها كمضادات فطرية ضد :

Candida albicans, Candida tropica, Aspergillus multi and Aspergillus niger.

أظهر معقد الكوبلت فعالية اكبر ضد الفطريات من مشتق البنزو ثايازول.

REFERENCES

- 1- Ed. S. Patai, "The chemistry of carbon-nitrogen double bond", John Wiley and Sons, New York, 1970, pp.61-146.
- 2- A. S. P. Azzouz, R. T. Ali, Synthesis of schiff bases derived from benzaldehyde and salicylaldehyde with some amino acids by a new develop method, *Russian J. of Coordination Chem.*, **30**, 9, 2004, pp. 655-659.

- 3- G.Valarmathy and R. Subbalakshmi, *Int. J. of Pharma and Bio Sciences*, **4**(3), 2013, pp. 287 – 295.
- 4- R. K. Dubey, C. M. Mishra , A. N. Mishra , Synthesis, Reactivity and Physico- Chemical Studies of Some Novel Mixed Ligand Complexes of Copper(II), *Indian J. of Chem.*, **44**, A, 2005, pp.1159-1164.
- 5- W. A. Al-Masoudi, H. Tooama and J. Hammed, Acute toxicity study of new dapsone Schiff base derivative in laboratory rats, *Bas.J.Vet.Res.*, Vol.1,No.1.2014.
- 6- A. Gupta and S. Rawat, Synthesis and Cyclization of Benzothiazole Review , *J. of Current Pharmaceutical Research*, **3**(1) 2010, p.13-23
- 7- A. Rana, N. Siddiqui, S. A. Khan, Benzothiazoles: A new profile of biological activities. *Indian J. Pharm Sci.*, **19** , 69, 2007, pp.10-17
- 8- A. Wayne, National Committee for Clinical Laboratory Standards, NCCLS Approved standard M27- PA, USA, 1997.
- 9- R. R. Coombs, M. K. Ringer, J. M. Blacquire, J. C. Smith, J. S. Neilsen, Palladium (II) Schiff base complexes derived from sulfanilamides and aminobenzothiazoles, *Transition metal chemistry*, **30**, 2005, pp. 411-418.
- 10- R. C. Maurya and P. Patel, Synthesis, magnetic and special studies of some novel metal complexes of Cu(II), Ni(II), Co(II), Zn(II),Nd(III) and U(VI)O₂ with Schiff base derived from sulfa drugs, *Spectroscopy Letters*, **32**(2), 1996, pp. 213-236.
- 11-S. Braun, H. Kalinowski and S.Berger, *(150 and More Basic NMR Experiments)* John Wiley & Sons, 1998, pp. 410.
- 12-Y. Zhang, V. Monga, and Y. Wang, Theoretical studies of the tautomers of pyridinethiones, *phys.Chem. A*, **112**, 2008, pp. 3231-3238.

- 13- W. A. Al-Masoudi, T. M. Al-Tememy, R. H. Al-Assadi, Computational study and antimicrobial activity of few Dapsone Schiff base derivatives, *European J. of Chemistry*, **5**, 2, 2014, pp. 351-355.
- 14-N. A. Ogorodnikova, On invariance of the Mulliken substituent-induced charge changes in quantum-chemical calculations of different levels, *J.Mol.Struct. THEOCHEM.*, 894, 2009, pp. 41-49.
- 15- A. Akbari and Alinia, Synthesis, characterization, and DFT calculation of a Pd(II) Schiff base complex, *Z. Turk.J.Chem.*, **37**, 2013, pp. 867-878.
- 16- Z. Zhou, R. G. Parr, Activation hardness: New index for describing the orientation of electrophilic aromatic substitution. *J. Am. Chem. Soc.*, **112**, 1990, pp. 5720-5724