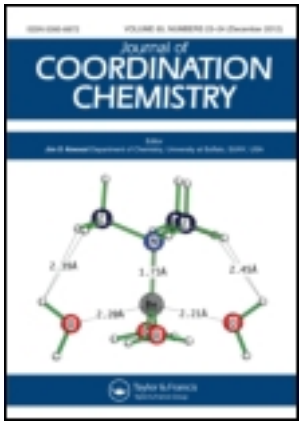

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Synthesis, spectral analysis, and molecular modeling of bioactive Sn(II)-complexes with oxadiazole Schiff bases

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Synthesis, spectral analysis, and molecular modeling of bioactive Sn(II)-complexes with oxadiazole Schiff bases

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Sn(II)-complexes of seven 2-amino-5-substituted-aryl-1,3,4-oxadiazole Schiff bases have been synthesized and characterized by various physico-chemical studies. Their structures have been confirmed by elemental analyses, infrared, ^{1}H NMR, UV-Vis, and mass spectral studies as well as thermal decomposition. Conductance measurements in methanol show these complexes to be non-electrolytes, and the molecular weight determinations support the proposed molecular formulae. The molecular structures of the complexes have been optimized by CS Chem 3-D Ultra Molecular Modeling and Analysis Program showing tetrahedral geometry. The bio-efficacy of the complexes has been examined against the growth of bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Aspergillus flavus* and *Candida albicans*) *in vitro* to evaluate their anti-microbial potential.

Keywords: Sn(II)-complexes; Oxadiazole Schiff bases; Molecular modeling; Antimicrobial activity

1. Introduction

Schiff bases are an important class of compounds reported to possess various biological activities [1], including antibacterial [2], antifungal [3], anti-inflammatory [4], antipyretic [5], antitumor [6, 7], and anticancer [8] activities. Therapeutic activities of coordination compounds offer a variety of geometries and reactivity for use in drug design [9]. Schiff bases are widely used as ligands in the synthesis of transition metal complexes [10, 11], due to their stability under a variety of oxidative and reductive conditions and due to the fact that imine ligands are borderline hard and soft Lewis bases [12]. Tin is extensively used as fungicides in crops, in food packaging, in some veterinary formulations, in wood preservation, as stabilizer for polyvinylchloride, as polymerization initiators and catalysts [13, 14]. In order to find cheap and effective antimicrobial agents, we report the synthesis, characterization, and molecular modeling of seven new Sn(II) complexes involving oxadiazole-based Schiff bases, SB_1–SB_7 (figure 1), and screening of these complexes for antimicrobial activities against bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Aspergillus flavus*).

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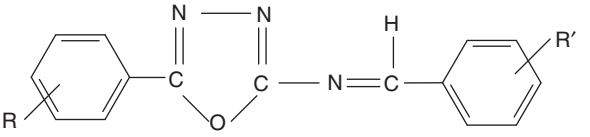


Figure 1. Structural formula of Schiff bases (SB_1–SB_7). R=R′=H (SB_1); R=H, R′=4-NO_2 (SB_2); R=3-Br, R′=3-Br (SB_3); R=3-Cl, R′=3-Cl (SB_4); R=4-F, R′=4-OH (SB_5); R=3-NO_2, R′=3-NO_2 (SB_6); R=4-OCH_3, R′=4-NO_2 (SB_7).

2. Experimental

2.1. *Materials and methodology*

All chemicals were of analytical grade and used as procured. The Schiff bases, SB_1–SB_7 (figure 1), were prepared by condensation of 2-amino-5-substituted-aryl-1,3, 4-oxadiazole (10 mmol) with substituted aryl aldehyde (10 mmol) in ethanol. These were recrystallized from the same solvent and dried *in vacuo*. Microanalyses (C, H, and N) were performed on a Perkin-Elmer 2400 elemental analyzer. Metal content was determined by EDTA titration after decomposition with nitric and perchloric acids [15]. Molar conductivities were measured in CH_3OH solutions, employing a conductance bridge of the type 523 conductometer at ambient temperature. Mass spectra were obtained using a Shimadzu LCMS-2010 eV mass spectrometer using the electron spray ionization (ESI) method at Gakushuin University (Japan). Infrared (IR) spectra from 4000 to 200 cm^{-1} were obtained as KBr discs with a Perkin-Elmer (model 1430) IR spectrophotometer at the Micro-analytical unit of Tanta University. A Bruker DMX 750 (500 MHz) spectrometer was used for obtaining ^1H NMR spectra, employing DMSO-d_6 as the solvent and TMS as the internal standard. Electronic spectra (in methanol) were taken on a Cary-400 double beam recording spectrophotometer. Heating of the complexes was performed in a muffle furnace at 100°C, 300°C, 500°C, and 750°C.

2.2. *Synthesis of tin(II) complexes*

To a weighed amount of anhydrous tin(II) chloride, the calculated amount of the Schiff bases was added in a 1 : 1 molar ratio using ethanol as the reaction medium. The color of the contents immediately changed and the resulting mixture was refluxed for 4–6 h on a water bath. The complex was precipitated, filtered off, and finally dried *in vacuo* over anhydrous calcium chloride after being repeatedly washed with hot water and ether to remove excess ligand and tin salt.

2.3. *Molecular modeling*

3-D molecular modeling of the structures of the complexes was performed using CS Chem 3-D Ultra Molecular Modeling and Analysis Program [16], an interactive graphics program that allows rapid structure building, geometry optimization with minimum energy, and molecular display. The program has the ability to handle transition metal complexes [17]. The correct stereochemistry was assured through

manipulation and modification of the molecular coordinates to obtain reasonable, low-energy molecular geometries. The potential energy of the molecule was the sum of the following terms:

$$E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele},$$

where all E's represent the energy values corresponding to the given types of interaction (Kcal mol^{-1}). The subscripts str, ang, tor, vdw, oop, and ele denote bond stretching, angle bonding, torsion, van der Waals interactions, out-of-plane bending and electronic interaction, respectively. The molecular mechanics describe the application of classical mechanics for the determination of molecular equilibrium structures. It enables the calculation of the total static energy of a molecule in terms of deviations from reference unstrained bond lengths, angles, and torsions plus non-bonded interactions. It has been found that off-diagonal terms are usually largest when neighboring atoms are involved, and so we have to take account of non-bonded interactions, but only between next-nearest neighbors [18].

2.4. *Biological activity: antibacterial and antifungal screening*

In vitro antibacterial and antifungal activities of the complexes against *E. coli*, *S. aureus*, *A. flavus*, and *C. albicans* were carried out using the modified Kirby–Bauer disc diffusion method [19] at the micro-analytical unit of Cairo University. Briefly, 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached 108 cells mL^{-1} for bacteria or 105 cells mL^{-1} for fungi [20]. Hundred microliters of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism was selected from primary agar plates and tested for susceptibility by the disc diffusion method [21]. From the many media available, NCCLS recommends Mueller–Hinton agar since it results in good batch-to-batch reproducibility. The disc diffusion method for filamentous fungi was tested using approved standard method (M38-A) which was developed by the NCCLS [22] for evaluating the susceptibilities of filamentous fungi to antifungal agents. The disc diffusion method for yeasts was developed using approved standard method (M44-P) by the NCCLS [23]. Plates were inoculated with filamentous fungi, *A. flavus*, at 25°C for 48 h; Gram (+) bacteria, *S. aureus*, and Gram (−) bacteria, *E. coli*. They were incubated at 35–37°C for 24–48 h and yeast as *C. albicans* was incubated at 30°C for 24–48 h, and then the diameters of the inhibition zones were measured in millimeters [24]. Standard discs of tetracycline (antibacterial agent) and amphotericin B (antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µL of solvent (distilled water, DMSO) were used as a negative control. The agar used is Meuller–Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well-documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper discs (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 µL of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical

infiltration around the disc. When an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition" or "clear zone". For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards [24]. Agar-based methods such as Etest and disc diffusion can be good alternatives because they are simpler and faster than broth-based methods [25, 26].

3. Results and discussion

All the complexes are air stable, colored, microcrystalline solids which are insoluble in water but soluble in methanol, ethanol, DMF, and DMSO. Composition and identity of the assembled compounds were deduced from elemental analyses, spectroscopic techniques (IR, UV-Vis, ^{1}H NMR, ESI-MS), and thermal studies. The analytical data of the complexes (table 1) indicated 1:1 metal to ligand stoichiometry. The satisfactory analytical data (table 1) and spectral studies revealed that the ligands and complexes were of good purity. Low values of molar conductance ($\Delta_M = 8.99$–$11.35\,\Omega^{-1}\,cm^2\,mol^{-1}$) show these complexes to be non-electrolytes [27].

3.1. *ESI-MS studies*

ESI-MS of $[SnL^1Cl_2]\cdot 4H_2O\cdot C_2H_5OH$, $[SnL^3Cl_2]$, $[SnL^4Cl_2]\cdot 5H_2O$, and $[SnL^7Cl_2]\cdot H_2O\cdot 2C_2H_5OH$ showed a molecular ion peak M^+ at m/z 557.0, 597.0, 598.0, and 623.9, respectively, that is equivalent to its molecular weight. The spectra of $[SnL^2Cl_2]\cdot$

Table 1. Physical properties and microanalyses of tin(II) complexes.[a]

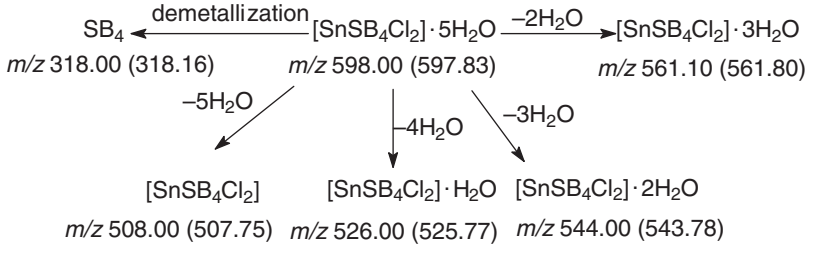
No.	Compound (Empirical formula)	M. wt. (Cal. M. wt.)	Color (Λ_m)	Elemental analysis %C	%H	%N	%M
1	$[SnSB_1Cl_2]\cdot 4H_2O\cdot C_2H_5OH$	557.0 (556.99)	Deep Yellow	36.24	4.66	7.84	21.54
	($C_{17}H_{25}Cl_2N_3O_6Sn$)		(10.11)	(36.66)	(4.52)	(7.54)	(21.31)
2	$[SnSB_2Cl_2]\cdot 6H_2O\cdot C_2H_5OH$	639.4 (638.03)	Reddish orange	31.76	4.74	6.24	18.25
	($C_{17}H_{28}Cl_2N_3O_{10}Sn$)		(11.35)	(32.00)	(4.42)	(6.59)	(18.60)
3	$[SnSB_3Cl_2]$	597.0 (596.66)	Faint yellow	34.60	2.82	7.51	19.61
	($C_{17}H_{15}Br_2Cl_2N_3O_2Sn$)		(10.42)	(34.22)	(2.53)	(7.04)	(19.89)
4	$[SnSB_4Cl_2]\cdot 5H_2O$	598.0 (597.83)	Deep yellow	31.34	2.95	7.45	19.69
	($C_{15}H_{19}Cl_4N_3O_6Sn$)		(8.99)	(30.14)	(3.20)	(7.03)	(19.85)
5	$[SnSB_5Cl_2]\cdot 6H_2O$	582.0 (580.95)	Buff	30.90	3.61	6.92	20.11
	($C_{15}H_{22}Cl_2FN_3O_8Sn$)		(9.23)	(31.01)	(3.82)	(7.23)	(20.43)
6	$[SnSB_6Cl_2]\cdot 3H_2O$	584.0 (582.91)	Red	31.32	2.42	12.45	20.80
	($C_{15}H_{15}Cl_2N_5O_8Sn$)		(11.24)	(30.91)	(2.59)	(12.01)	(20.36)
7	$[SnSB_7Cl_2]\cdot H_2O\cdot 2C_2H_5OH$	623.9 (624.04)	Brown	38.81	3.86	9.15	19.44
	($C_{20}H_{26}Cl_2N_4O_7Sn$)		(9.84)	(38.49)	(4.20)	(8.98)	(19.02)

The yield for the synthesized complexes was 80–82%.
M. Wt. = molecular weight obtained from mass spectral measurements; (Cal. M. Wt.) = calculated molecular weight; Λ_m = molar conductance ($\Omega^{-1}\,cm^2\,mol^{-1}$).
[a]All the synthesized complexes decompose with out melting above 280°C.

$6H_2O \cdot C_2H_5OH$, $[SnL^5Cl_2] \cdot 6H_2O$, and $[SnL^6Cl_2] \cdot 3H_2O$ displayed peaks at *m/z* 639.4, 582.0, and 584.0, respectively, corresponding to $[M]^+ + 1$. Tin(II) complexes (**1**–**7**) decompose *via* abstraction of the ligand, which give rise to the presence of molecular ion peaks attributable to $[L]^+$, besides other peaks due to different fragmentation processes of the complexes (scheme 1). This is a common behavior for metal ion complexes containing different ligands (ML) which decompose during spray ionization through cleavage of the metal-ligand bond [28, 29]. For example, **4** gave fragment ion peaks *m/z* 561.1, 544.0, 526.0, 508.0, and 318.0, due to loss of two, three, four, five water molecules, and L^4, respectively (scheme 1). The results of mass spectra are in satisfactory agreement with elemental analyses confirming the constitutions and purities of the prepared Sn(II) complexes (table 1).

3.2. *IR spectral studies*

Comparison of IR spectra of the ligands with those of their complexes (table 2) resulted in the following information regarding various groups. IR spectra of the Schiff bases exhibited strong bands at 1602–1591 cm^{-1}, assigned to ν(C=N) of azomethine.



Scheme 1. Fragmentation pathways of $[SnSB_4Cl_2] \cdot 5H_2O$.

Table 2. IR and ^{1}H NMR and UV-Vis spectral data of the Schiff bases (SB_1–SB_7) and their Sn(II) complexes.

	IR spectra (cm^{-1})					^{1}H NMR spectra (ppm)		
Complex	$\nu(H_2O)$ or ν(EtOH)	ν(C=N) (ring)	ν(C=N) (imine)	ν(M–N)	ν(M–Cl)	$\delta_{Ar-\underline{H}}$	$\delta_{\underline{CH}=N}$	UV-Vis spectra
SB_1	–	1652	1591	–	–	7.24–7.99	10.23	207, 277
1	3301	1657	1601	397	326	7.35–7.80	–	213, 283
SB_2	–	1647	1597	–	–	6.47–7.98	10.24	211, 272
2	3397	1657	1599	395	329	7.22–7.80	10.16	217, 280
SB_3	–	1651	1597	–	–	7.31–7.87	10.01	207, 281
3	3293	1662	1598	419	308	7.33–7.89	10.22	226, 292
SB_4	–	1653	1596	–	–	7.32–7.89	8.68	204, 283
4	3295	1654	1597	438	321	7.33–7.75	10.32	219, 295
SB_5	–	1662	1597	–	–	6.28–8.04	9.77	206, 276
5	3317	1654	1595	360	289	7.24–7.84	9.78	217, 282
SB_6	–	1646	1598	–	–	6.50–7.98	10.45	211, 267
6	3408	1662	1580	418	307	7.43–8.31	10.40	215, 272
SB_7	–	1658	1602	–	–	7.03–8.39	10.13	214, 273
7	3318	1659	1605	379	302	7.04–8.38	10.14	216, 285

In comparison with spectra of the Schiff bases, all Sn(II) complexes exhibited this band at 1605–1580 cm^{-1}; the shift of this band indicates that azomethine nitrogen is coordinated [30]. In addition, the band at 1662–1646 cm^{-1} in the Schiff bases due to ν(C=N) group of the oxadiazole ring shifted by 5–16 cm^{-1} in complexes, confirming participation of C=N of ring in coordination with tin [31]. The new bands at 438–360 cm^{-1} in spectra of the complexes are assigned to Sn–N stretching frequencies [32]. Another new band at 329–289 cm^{-1} is ascribed to Sn–Cl bond formation [33]. Thus the IR spectral results provide strong evidence for bidentate Schiff bases. The broad bands at 3408–3295 cm^{-1} in spectra of hydrated complexes are assigned to ν(OH) of water or ethanol attached to tin(II).

3.3. *^{1}H NMR spectral studies*

^{1}H NMR spectra of the Schiff bases and their tin(II) complexes are collected in table 2. The signal for the azomethine proton at 8.68–10.45 ppm in the ligands shifts downfield in spectra of the tin(II) complexes. This de-shielding is probably due to donation of the lone pair of electrons on the azomethine nitrogen to tin, resulting in the formation of a coordinate bond [34]. The multiplets observed at 6.28–8.39 ppm in spectra of SB_1–SB_7 due to aromatic protons have downfield shifts in spectra of the complexes, attributed to increased conjugation upon complex formation [29, 35]. Thus the ^{1}H NMR results support the IR inferences.

3.4. *Electronic spectral studies*

Electronic absorption spectra of the Schiff bases and their Sn(II) complexes were recorded in methanol (table 2). The spectra of the free ligands exhibit two absorptions at 204–214 and 277–289 nm assigned to high energy π–π^* transition corresponding to the ^1L_a–^1A in the aromatic moiety [36] and n–π^* transition within the CH=N [37], respectively. The shift of these bands to longer wavelength in spectra of all Sn(II) complexes is a good evidence of complex formation [38]. It has been reported that the metal is capable of forming dπ–pπ bonds with ligands containing nitrogen as donor. Tin has its 5d orbital completely vacant and, hence, N→Sn bonding can take place by the acceptance of the pair of electrons from nitrogen of the ligand. Sn→N bonding can occur by partial transfer of electrons of the 4d orbitals of the stannous ion to the vacant p-orbitals of nitrogen of the ligand [34].

3.5. *Thermal decomposition studies*

Complexes **1–7** were heated at four temperatures (100°C, 300°C, 500°C, and 750°C) in a muffle furnace for 20–30 min. The resulting weights were measured. The weight loss at 100°C corresponds to loss of lattice water or ethanol from the complexes [39]. No measured weight loss after heating at 300°C indicates that the complexes do not contain coordinated water (table 1). On heating at 500°C, the weight indicates loss of parts of the ligand. The weight of the pyrolysis product after heating at 750°C corresponds to tin oxide [40].

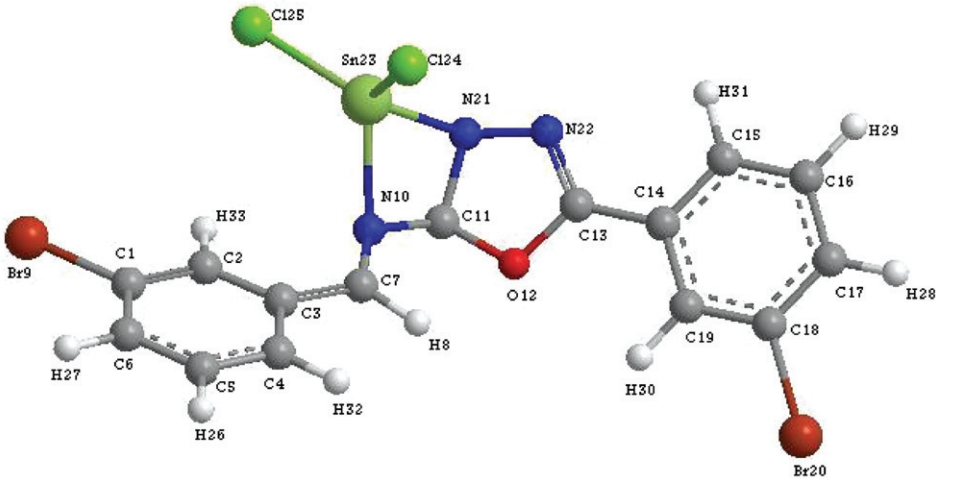


Figure 2. 3-D molecular modeling of complex $[SnSB_3Cl_2]$ (**3**).

3.6. *3-D molecular modeling and analysis of bonding modes*

Molecular mechanics attempts to reproduce molecular geometries, energies, and other features by adjusting bond length, bond angles, and torsion angles to equilibrium values that are dependent on the hybridization of an atom and its bonding scheme. In order to obtain estimates of structural details of these four-coordinate complexes, we have optimized the molecular structure of $[SnSB_3Cl_2]$ (**3**) as a representative compound. For convenience, the atoms are numbered in Arabic numerals (figure 2). The details of bond lengths and bond angles per the 3-D molecular structure are given in "Supplementary material". Energy minimization was repeated several times to find the global minimum [40].

The energy minimization value for tetrahedral and without restricting the structure for **3** is almost the same, i.e., 42.8472 Kcal mol^{-1}. The tetrahedral bond lengths are Sn(23)–N(21) 2.095, Sn(23)–Cl(24) 2.383, Sn(23)–Cl(25) 2.382, and N(10)–Sn(23) 2.099 Å, respectively. The molecular modeling for the modulated complex (figure 2 and Supplementary material) shows the angles around tin are 66.987°, 113.978°, 114.147°, 115.390°, 116.064°, and 119.300°; these angles do not change for the other complexes, indicating distorted tetrahedral geometry around Sn(II) [16, 17, 41]. Thus the proposed structure of **3** as well as of the others is acceptable.

3.7. *Antimicrobial activity*

The *in vitro* anti-microbial activities of the compounds were tested against *E. coli*, *S. aureus*, *S. faecalis*, and *C. albicans* by the modified Kirby–Bauer disc diffusion method [19]. Standard drugs tetracycline and amphotericin B were also tested for their antibacterial and antifungal activities at the same concentration and conditions of the tested compounds. The complexes had significant antimicrobial activities against the tested organisms (table 3). Complexes **1**, **2**, **6**, and **7** exhibited high activity against most of the tested bacteria and fungi, whereas **3**, **4**, and **5** are less active against tested

Table 3. Antimicrobial activities of **1–7**.

Compound	Inhibition zone diameter (mm mg^{-1} sample)			
	E. coli (G^+)	*S. aureus* (G^-)	*A. flavus* (Fungus)	*C. albicans* (Fungus)
Control: DMCL	0.0	0.0	0.0	0.0
Tetracycline (antibacterial agent)	33.0	30.0	–	–
Amphotericin B (antifungal agent)	–	–	20.0	20.0
Complex **1**	13.0	14.0	0.0	13.0
Complex **2**	14.0	15.0	0.0	14.0
Complex **3**	12.0	11.0	0.0	0.0
Complex **4**	11.0	11.0	0.0	0.0
Complex **5**	12.0	12.0	0.0	0.0
Complex **6**	13.0	15.0	11.0	14.0
Complex **7**	13.0	15.0	16.0	14.0

bacteria and are completely inactive against tested fungi. Compared with standard drugs (tetracycline and amphotericin B) the complexes were less active. The data prove the potential of **1**, **2**, **6**, and **7** as broad spectrum antimicrobial agents. The activity of any compound is a complex combination of steric, electronic, and pharmacokinetic factors. The action of the compounds may involve the formation of a hydrogen bond through −N=C of the chelate or the ligand with the active centers of the cell constituents resulting in interference with normal cell process [18]. The microbotoxicity of the compounds may be ascribed to the metal ions being more susceptible toward the bacterial cells than ligands [42]. The tin(II) complexes (table 3) with oxadiazole Schiff bases (**1–7**) have higher antimicrobial activity compared to other tin(II) complexes with ethylenediamine Schiff bases [18].

4. Conclusion

The coordination complexes of Sn(II) with seven new bidentate 2-amino-5-substituted-aryl-1,3,4-oxadiazole Schiff bases (SB_1–SB_7) were synthesized and characterized by elemental, thermal analyses, spectral, and magnetic data. The IR, ^{1}H NMR, and UV-Vis spectra revealed that SB_1–SB_7 coordinate bidentate to tin(II) through nitrogen-forming tetrahedral complexes. Mass spectra under EI-conditions are in agreement with elemental analyses confirming the constitutions and purities of the Sn(II) complexes. The proposed structures of the investigated complexes were supported by 3-D molecular modeling of **3** as a representative compound. The synthesized complexes were active against bacteria (*E. coli* and *S. aureus*) and fungi (*A. flavus* and *C. albicans*), thus giving a new thrust of these compounds in the field of metallo-drugs (bio-inorganic chemistry).

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