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Solvent-free synthesis, characterization, biological activity of schiff bases and their metal (II) complexes derived from ferrocenyl chalcone

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ABSTRACT

A novel efficient method for the synthesis of ferrocenyl chalcone Schiff bases and their metal (II) complexes has been developed. Schiff bases were synthesized with TsOH (*p*-toluenesulfonic acid) as catalyst and complexed with metal (II) salts (Zn (II), Pb (II), Cd (II), Ni (II)). The compounds were characterized by various spectroscopic techniques and elemental analysis. The thermal stability of the complexes was performed by thermogravimetric analysis (TGA). In addition, The ligands and their metal complexes have been screened in vitro antibacterial (*S. aureus, Streptococcus, Actinomycete, E. coli and P. aeruginosa*), antifungal properties (*C. albicans, A. fumigatus, A. niger, A. flavus, S. cerevisiae*). The results revealed that Zn (II) complexes (H1, H5, H9) were the most active against all bacterial and fungal strains.

1. Introduction

Schiff base has the important biological and chemical significance [1] due to the presence of −C=N-, easing to coordinate and ring. Schiff base and its metal complexes [2,3] have numerous potential applications in the fields of medicine, catalysis, materials and analytical titration [4–10], attracting scientists to conduct research in these areas. Among them, the organic complexes containing ferrocenyl have attracted much attention in biological mechanism modeling, anti-cancer, anti-bacterial, anti-viral and so on [11–13].

Up to now, ferrocenyl schiff base has been developed. H.Ye et al. [14] have synthesized ferrocene-based Schiff base metal (II) complexes catalyzed by phosphorus oxychloride. A.Abou- Hussein et al. [15] have reported the synthesis of ferrocene-based Schiff base complexes and their antimicrobial activities against *Pseudomonas, Fusarium oxysporum, Staphylococcus aureus.* Furthermore, our research team has synthesized ferrocene-based Schiff base metal (II) complexes which exhibited excellent antibacterial activities [16].

However, the reported methods for synthesizing ferrocene-based Schiff bases were liquid-phase method with disadvantages of environmental pollution, long reaction time, complicated post-treatment, low yield and so on [17–20]. In the current study, the solvent-free method avoided these problems and it was green, simple, economical [21,22]. In this paper, a series of Schiff bases and their metal (II) complexes were synthesized by solvent-free method in search of new antibacterial and antifungal bioorganometallics.

2. Experimental

2.1. Materials and methods

All chemicals were analytical grade and used without further purification. All reagents were provided from Aldrich and Sigma chemicals companies. The melting points were determined in an open glass capillary and uncorrected. The C, H, N, S analyses were performed with a Fisons EA 1108 (CHNS−O) elemental analyzer. Infrared spectra (400-4000 cm^{-1}) were recorded on Bruker Vector-22 FT-IR with samples prepared as KBr pellets. The NMR tests were run on Bruker Avance-400 MHz in $CDCl_3$ or DMSO-d_6 solution with TMS as an internal standard. The molar conductance of the complexes were measured with 1×10^{-3} M DMSO solutions at 25 ± 1 °C using a systronic conductivity bridge type 305.

The ferrocenyl chalcones were prepared as reported [23,24].

2.2. Synthesis of ferrocenyl chalcone schiff base

In a dry agate mortar, the ferrocenyl chalcone (1 mmol), *S*-

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benzyl dithiocarbazate (1.2 mmol) and TsOH (1.2 mmol) were ground at room temperature. The progress of the reaction was monitored by TLC until completed. And the mixture was incubated in an oven at 80 °C for 1 h. Then, The products were washed with water, filtered through buchner funnel, recrystallized from ethanol and dried (Scheme 1).

2.3. Synthesis of the metal (II) complexes

Among all Schiff bases, L1, L6, L11 are representative compounds containing electron withdrawing and electron donating substituents. They were selected to synthesize the novel metal (II) complexes with $M(OAc)_2 \cdot 2H_2O$.

In a dry agate mortar, *S*-benzyl-N-(1-ferrocenyl -3-arylacrylic acid ketone) dithiocarbazinate (2 mmol), $M(OAc)_2 \cdot 2H_2O$ (1 mmol) and TsOH (1.2 mmol) were ground at room temperature, monitoring by TLC until completed. The mixture was washed with water, filtered through buchner funnel, recrystallized from acetone and dried (Scheme 1).

2.4. Biological activity

2.4.1. Antibacterial activity (in vitro)

To assess the biological potential of the synthesized compounds, they were tested by different species of bacteria. The organisms used in the present investigations included three Gram-positive bacterium (*S. aureus* ATCC 9144, *Streptococcus* BNCC 1022637 and *Actinomycetes* ATCC 55605) and two Gram-negative bacterium (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 43288). The antimicrobial activities were evaluated at a concentration of 3 g/L. The bacterial inoculum was coated on the surface of the nutrient agar with the sterile cotton swab. Tetracycline was set as the standard antibacterial drug, DMSO as a control solvent and the plates were incubated overnight at 37 °C. The results of the antimicrobial activities were showed in Fig. 5.

2.4.2. Antifungal activity (in vitro)

The antifungal activities of compounds were tested against five fungal strains (*C. albicans, A. flavus, A. niger* ATCC9092, A. fumigatus ATCC 46645, *S. cerevisiae* ATCC 87358) in vitro. And it was cultured on malt medium about 48 h, with filtering of the culture through a thin layer of sterile sintered glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. For preparation of plates and inoculation, 1.0 mL of inoculum was added to 50 mL agar medium (50 °C) and mixed. The agar was poured into the 120 mm Petri dish and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using a proper sterile tube. Then, fill wells were filled up to the surface of agar with 0.1 mL of the tested compounds dissolved in DMSO (200 μmol/mL). The plates were left on a leveled surface, and incubated at 30 °C for 48 h. The results were recorded as the diameter of the inhibition zones and compared with the standard drug Nystatin.

2.4.3. Minimum inhibitory concentration (MIC)

Compounds with the high antimicrobial activity (≥20 mm) were selected for minimal inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using disk diffusion techniques, and compounds containing 10, 25, 50, and 100 μg/mL were prepared and applied.

3. Results and discussion

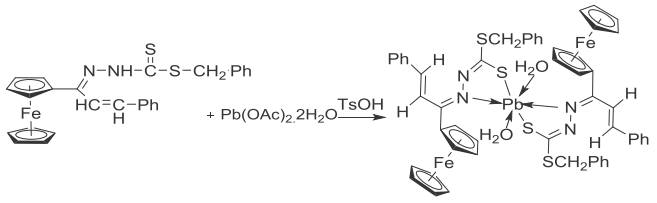
3.1. Optimization of reaction conditions

As is known to all, the liquid-phase method has the disadvantages with the great environmental pollution, long reaction time and so on. Instead, the solvent-free method is the green method with mild conditions, shorter time, higher yield and so on. Taking the reaction of *S*-benzyl-N-(1-ferrocenyl-3- propiophenone) dithiocarba-zinate (L1) and $Pb(OAc)_2 \cdot 2H_2O$ as an example, optimizing the reaction conditions (Scheme 2), the results were shown in Table 1.

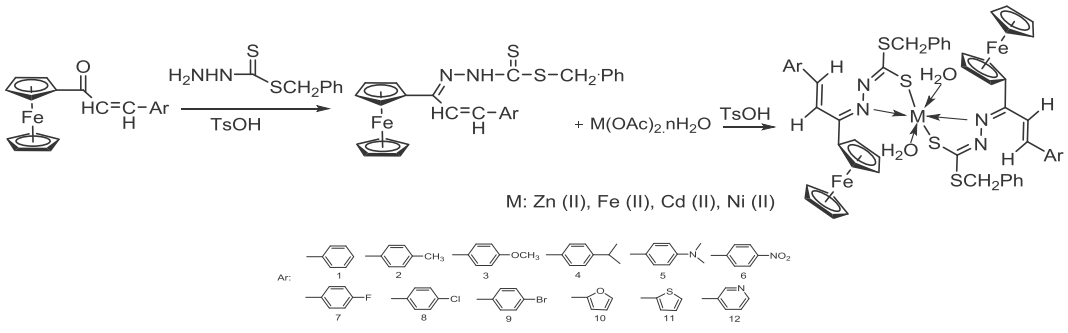
Under the solvent-free method, the catalysts were: $SiO_2 \cdot SO_3H$, silica gel, neutral Al_2O_3, BSA (benzenesulfonic acid), TsOH and DBSA (dodecyl-benzenesulfonic acid), no catalyst as a blank experiment (Table 1). The yield of the catalyst used was higher than the blank experiment. And the highest yield was up to 90.1% under the catalyst of TsOH with the dosage 1:1.2 (Table 1, entry 8).

Schiff base was synthesized from *S*-benzyl dithiocarbazate and different ferrocenl chalcone through the Nucleophilic addition-elimination reaction. Extending the substrate of the reaction and comparing the yields of compounds under the optimum conditions, they were synthesized by liquid-phase and solvent-free method, respectively. For different Schiff bases and metal complexes, the yields by the solvent-free method were higher than the liquid-phase method (Fig. 1, Fig. 2).

Among them, the yields of the Schiff bases having strong



Scheme 2. Synthesis of H2.



Scheme 1. Synthesis of Schiff bases and their metal (II) complexes.

Table 1
Optimization of reaction conditions.

Entry	Comp. method	Solvent	Catalyst	Catalyst dosage[c]	Temperature (°C)	Time	Yield (%)[d]
1	Liquid-phase[a]	ethanol	HAc	1:1.2	78	6 h	70.8
2		ethanol	TsOH	1:1.2	78	4 h	75.2
3	Solvent-free[b]	–	–	–	25	60 min	10.4
4		–	$SiO_2 \cdot SO_3H$	1:1.2	25	25 min	44.7
5		–	Silica gel	1:1.2	25	24 min	43.3
6		–	Neutral Al_2O_3	1:1.2	25	18 min	54.6
7		–	BSA	1:1.2	25	21 min	57.8
8		–	TsOH	1:1.2	25	10 min	90.1
9		–	DBSA	1:1.2	25	35 min	51.5
10		–	TsOH	1:1.1	25	10 min	84.8
11		–	TsOH	1:1.3	25	10 min	90.0

[a] Reaction conditions: S-benzyl-N-(1-ferrocenyl-3-propiophenone)dithiocarbazinate L1 (1 mmol), catalysts of glacial acetic acid or TsOH (1.2 mol) and $Pb(OAc)_2 \cdot 2H_2O$ (2 mol) in solvent by refluxing.
[b] Reaction conditions: S-benzyl-N-(1-ferrocenyl-3-propiophenone)dithiocarbazinate L1 (1 mmol), $Pb(OAc)_2 \cdot 2H_2O$ (2 mol) and TsOH (1.2 mmol) in dry mortar by grinding at room temperature.
[c] S-benzyl-N-(1-ferrocenyl-3-propiophenone)dithiocarbazinate L1 to catalyst.
[d] Isolated yield.

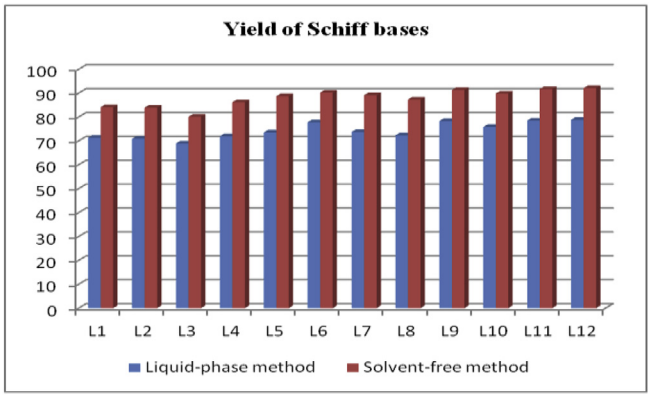


Fig. 1. Comparison the yield of Schiff bases.

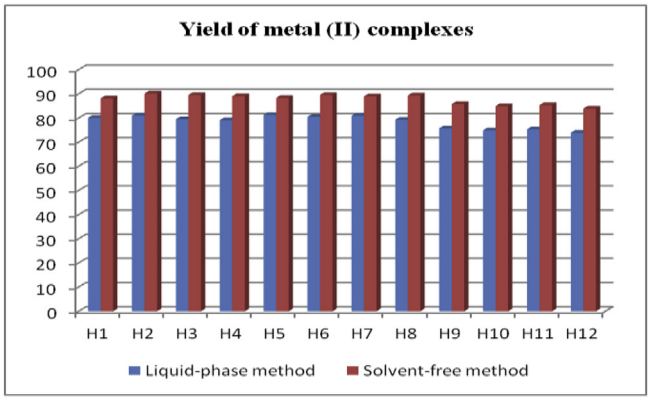


Fig. 2. Comparison the yield of the (II) metal complexes.

electron-withdrawing groups (-OCH_3, $-N(CH_3)_3$, $-NO_2$, −F, -Cl, -Br) were higher than others, making carbonyl carbon lower electron density and more beneficial to attack the nucleophiles.

Synthesis of Schiff bases and their metal (II) complexes

3.2. Solubility of schiff bases and their metal (II) complexes

The obtained Schiff bases were stable in the air, soluble in solvents such as chloroform, DMF, DMSO and acetone, slightly soluble in methanol, absolute ethanol and isopropanol, but insoluble in diethylether, water (Table 2). The Schiff bases (L1, L6, L11) were complexed with Zn^{2+}, Pb^{2+}, Cd^{2+} and Ni^{2+} metal ions (using the metal (II) salts: $Zn(OAc)_2 \cdot 2H_2O$, $Pb(OAc)_2 \cdot 2H_2O$, $Cd(OAc)_2 \cdot 2H_2O$, $Ni(OAc)_2 \cdot 2H_2O$) to obtain the complexes (H1−H12). They were stable in the air, insoluble in solvents such as methanol, absolute ethanol, diethylether, isopropanol and water, soluble in chloroform, DMF, DMSO and acetone.

3.3. Reusability of catalyst

The reusability of catalyst is one of the most significant properties for the industrial applications and environment. The catalyst (TsOH) was separated from the reaction system by filtration and used for the next experiments. Taking the reaction of *S*-benzyl-N-(1-ferrocenyl-3-benzeneacrylketone) dithiocarbazate (L1) and $Pb(OAc)_2 \cdot 2H_2O$ as an example (Scheme 2), the reusability of catalyst was investigated at the same reaction condition. It was found that TsOH was recycled five times and the yield was loss less (Fig. 3).

3.4. Structural analysis

3.4.1. IR spectrum

The IR spectra of the Schiff base was compared with its complexes. The IR absorption peaks between 1501 and 1599 cm^{-1} could be assigned to the C=N stretching vibration. The *v* (C=N) absorption peak of the complexes was weakened, and the *v* (C=S) was shifted from 1068 cm^{-1} to 1035 cm^{-1} *v* (C-SM) (the peak represented complexes). All the changes indicated that Schiff base existed tautomeric forms during the complexes formation. The N atom and the S atom were coordinated with the metal (II) ions after the proton was lost, indicating the formation of complexes.

3.4.2. ^1H NMR spectrum

The Schiff bases ligands and their metal (II) complexes were further characterized by ^1H NMR. The ^1H NMR spectrum of the Schiff base ligands exhibited a single peak around δ 12.20 ppm (s, 1H), disappeared after exchanged of the heavy water and attributed to the N−H proton. The other protons of the cyclopentadienyl ring without substituents appeared near 4.33 ppm (s, 5H), and the cyclopentadienyl ring with monosubstituted appeared near 4.61 ppm (s, 2H) and 4.94 ppm (s, 2H), respectively. In addition, the peak of O−H proton disappeared in complexes, indicating the successful coordination of metal (II).

Table 2
Solubility of Schiff bases and their metal (II) complexes.

Solvent	Solubility[a]														
	L1	H1	H2	H3	H4	L2	H5	H6	H7	H8	L3	H9	H10	H11	H12
Methanol	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS
Ethanol	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS
Diethylether	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
Isopropanol	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS	SS	IS	IS	IS	IS
Water	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS
Chloroform	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMF	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMSO	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Acetone	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a] S-Soluble, SS-Slightly soluble, IS-Insoluble.

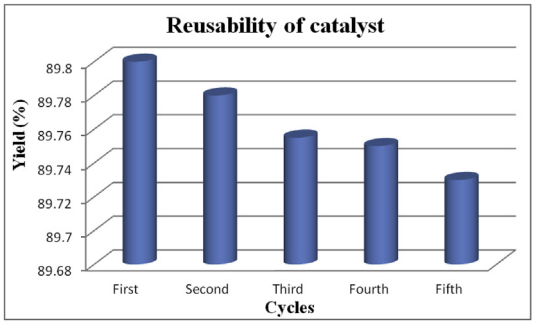


Fig. 3. Reusability of catalyst for the metal (II) complexes.

3.4.3. Molar conductance measurements

The molar conductivity of Schiff bases and their metal (II) complexes were measured at room temperature in a concentration of 1×10^{-3} M DMSO. The results were listed in Table 3, ranging from 5.89 to 8.09 Ω^{-1} mol^{-1} cm^2. It showed that Schiff bases had not the molar conductivity, and their metal complexes had the lower molar conductivity ($\Lambda m<50$), indicating nonelectrolytic nature of the complexes.

3.4.4. Thermogravimetric analysis of complexes

TGA-DTA analysis of complex H5 ($[Zn(L6)_2]\cdot 2H_2O$) was done in nitrogen atmosphere at a heating of 10 °C/min over a temperature range of 600 °C(Fig. 4).

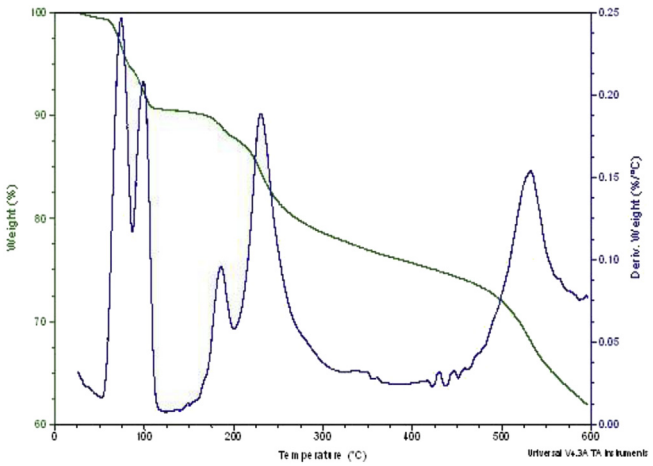


Fig. 4. The TGA spectrum of H5.

The main skeleton began to decompose with increasing of temperature. It was due to unstable and decomposed cyclopentadienyl ring. The thermal behavior of other complexes was similar to H5 and experienced a series of weightlessness process.

3.5. Biological activity

3.5.1. Antibacterial activity (in vitro)

The mode of action of the compounds might involve formation

Table 3
Elemental analysis, molar conductivity, molar weight and melting point of the metal (II) complexes.

Compounds	Formula, molar weight	Color	Yield (%)	Found (calcd.) %			M.P. (°C)	Λm ($\Omega^{-1}mol^{-1}cm^2$)
				C	H	N		
L1	$C_{27}H_{24}FeN_2S_2$ 496.47	Brown	84.1	65.13 (65.31)	4.92 (4.87)	5.41 (5.64)	118–120	–
H1	$C_{54}H_{50}ZnFe_2N_4S_4O_2$ $[Zn(L1)_2]\cdot 2H_2O$, 1092.34	Red	88.1	61.47 (61.41)	4.45 (4.39)	5.41 (5.31)	178–180	6.36
H2	$C_{54}H_{50}PbFe_2N_4S_4O_2$ $[Pb(L1)_2]\cdot 2H_2O$, 1234.15	Red	90.1	54.23 (54.14)	3.96 (3.87)	4.76 (4.68)	169–171	5.89
H3	$C_{54}H_{50}CdFe_2N_4S_4O_2$ $[Cd(L1)_2]\cdot 2H_2O$, 1139.36	Red	89.5	58.87 (58.79)	4.31 (4.20)	5.21 (5.08)	173–175	6.96
H4	$C_{54}H_{50}NiFe_2N_4S_4O_2$ $[Ni(L1)_2]\cdot 2H_2O$, 1085.64	Red	89.0	61.83 (61.80)	4.52 (4.42)	5.41 (5.34)	183–185	7.01
L6	$C_{27}H_{23}FeN_3S_2O_2$ 541.47	Brown	90.2	59.93 (59.89)	4.21 (4.28)	7.81 (7.76)	143–145	–
H5	$C_{54}H_{48}ZnFe_2N_6S_4O_6$ $[Zn(L6)_2]\cdot 2H_2O$, 1182.34	Red	88.3	56.67 (56.59)	3.95 (3.87)	7.41 (7.33)	199–201	7.13
H6	$C_{54}H_{48}PbFe_2N_6S_4O_6$ $[Pb(L6)_2]\cdot 2H_2O$, 1324.15	Red	89.5	50.43 (50.36)	4.52 (3.44)	6.61 (6.53)	190–192	5.96
H7	$C_{54}H_{48}CdFe_2N_6S_4O_6$ $[Cd(L6)_2]\cdot 2H_2O$, 1229.36	Red	88.9	54.43 (54.36)	3.81 (3.72)	7.14 (7.05)	203–205	7.52
H8	$C_{54}H_{48}NiFe_2N_6S_4O_6$ $[Ni(L6)_2]\cdot 2H_2O$, 1175.64	Red	89.3	56.98 (56.92)	3.92 (3.89)	7.41 (7.38)	204–206	6.86
L11	$C_{25}H_{22}FeN_2S_3$ 502.49	Brown	91.7	59.64 (59.75)	4.57 (4.41)	5.65 (5.58)	123–125	–
H9	$C_{50}H_{46}ZnFe_2N_4S_6O_2$ $[Zn(L11)_2]\cdot 2H_2O$, 1104.40	Red	85.7	58.27 (58.19)	3.85 (3.71)	5.49 (5.43)	158–160	7.25
H10	$C_{50}H_{46}PbFe_2N_4S_6O_2$ $[Pb(L11)_2]\cdot 2H_2O$, 1246.21	Red	84.9	51.23 (51.16)	3.32 (3.26)	4.86 (4.77)	149–151	6.31
H11	$C_{50}H_{46}CdFe_2N_4S_6O_2$ $[Cd(L11)_2]\cdot 2H_2O$, 1151.42	Red	85.3	55.74 (55.66)	3.63 (3.55)	5.26 (5.19)	162–164	7.56
H12	$C_{50}H_{46}NiFe_2N_4S_6O_2$ $[Ni(L11)_2]\cdot 2H_2O$, 1097.70	Red	83.9	58.65 (58.57)	3.83 (3.74)	5.51 (5.47)	173–175	7.14

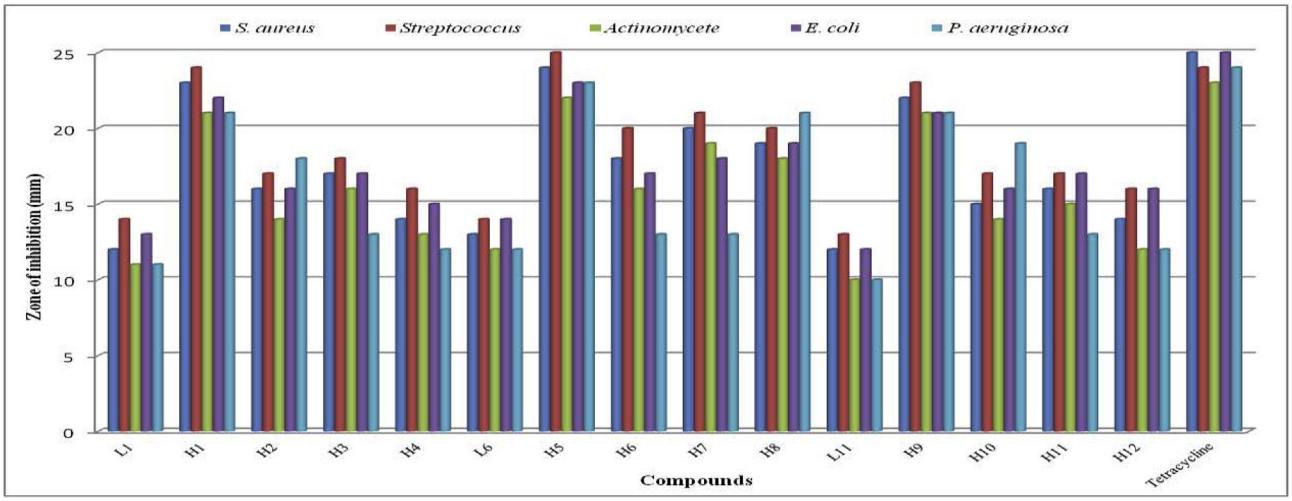


Fig. 5. Comparison of antibacterial activity of ligands (L1, L6, L11) and their metal (II) complexes.

of a hydrogen bond through the azomethine group (>C=N-) with the active centers of various cellular constituents, resulting in interference with normal cellular processes [25]. It had been suggested that the ligands with nitrogen and oxygen donor systems, inhibiting enzyme activity. The enzymes required these activity groups which appeared to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduced the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [26] within the chelate ring system formed during coordination. Conversely, this process increased the lipophilic nature of the central metal atom, which favored its permeation more efficiently through the lipid layer of the microorganism [27], destroying them more aggressively.

It could be seen that all compounds showed different antibacterial activities compared with the standard drug Tetracycline (Fig. 5). Among the three Gram-positive bacteria, the compounds L1, L6, L11, H4, H12 showed weak activities against *S. aureus*, while the compounds H2, H3, H6, H7, H8, H10, H11 showed moderate activities. All compounds showed the highest activities against *Streptococcus* and the weakest activities against *Actinomycetes*. Among the two Gram-negative bacteria, the compounds H1, H5, H9 showed significant activities against *E. coli*, while compounds L1, L6, L11 showed weak activity. The compounds H1, H5, H9, H8 showed significant activity against *P. aeruginosa*. In short, The Zn (II) complexes (H1, H5, H9) exhibited significant activities against all bacterial strains.

3.5.2. Antifungal activity (in vitro)

Metal ions were adsorbed on the cell walls of the microorganisms, disturbing the respiration processes of the cells and thus blocking the protein synthesis that was required for further growth of the organisms. Hence, metal ions were essential for the growth-inhibitory effects [28]. The lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials, lipophilicity is an important factor controlling the antifungal activity. Upon chelation, the polarity of the metal ion would be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. This increased lipophilicity facilitated the penetration of the complexes into lipid membranes, further restricting proliferation of the microorganisms.

The results showed that most compounds had the great

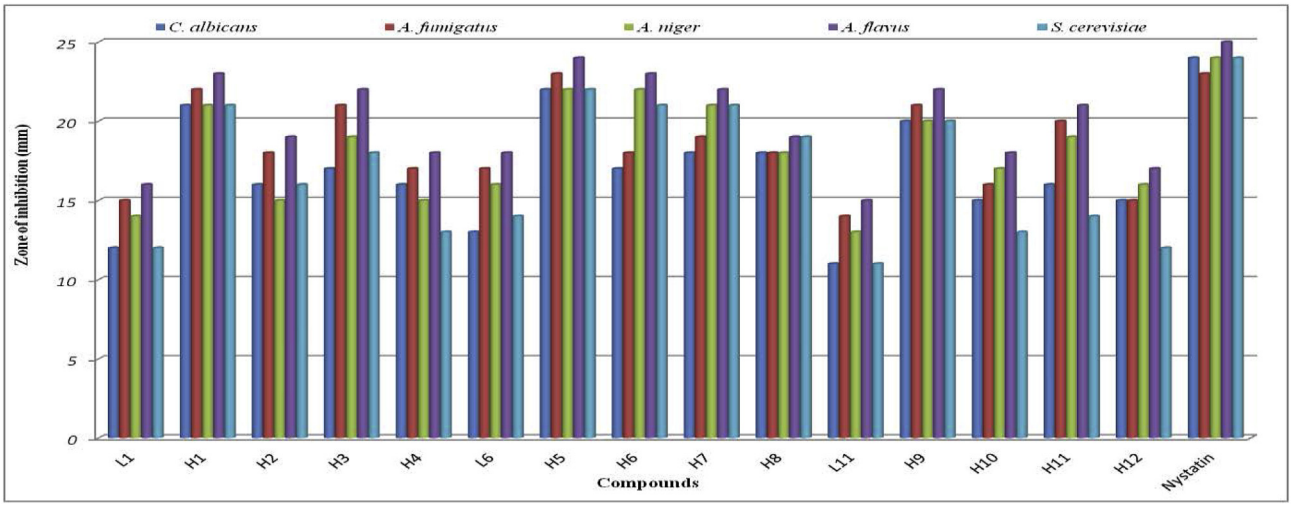


Fig. 6. Comparison of antifungal activity of ligands (L1, L6, L11) and their metal (II) complexes. (concentration used 1 mg/mL of DMSO) of the ligands and their metal (II) complexes [zone of inhibition (mm)].

Table 4
Minimum inhibitory concentration (M/mL) of the selected compounds H1, H5 and H9 against bacteria and fungi.

Bacteria/Fungi	H1	H5	H9
S. aureus	1.349×10^{-8}	1.268×10^{-7}	–
Streptococcus	2.713×10^{-8}	–	3.121×10^{-7}
Actinomycete	2.311×10^{-8}	–	2.871×10^{-7}
E. coli	1.893×10^{-7}	1.351×10^{-8}	3.582×10^{-7}
P.aeruginosa	1.213×10^{-7}	–	3.785×10^{-7}
C. albicans	2.122×10^{-7}	1.471×10^{-8}	2.531×10^{-7}
A. fumigatus	1.538×10^{-8}	1.891×10^{-8}	1.334×10^{-8}
A. niger	2.301×10^{-8}	1.521×10^{-8}	3.273×10^{-8}
A. flavus	1.205×10^{-8}	–	1.312×10^{-8}
S. cerevisiae	1.415×10^{-8}	–	1.571×10^{-8}

antifungal activities against different fungal strains and the activities of all ligands were enhanced after complexation(Fig. 6). The compounds H1, H3, H5, H9, H11 showed excellent antifungal activities against *A. fumigatus*. The compounds H1, H5, H6, H7, H9 showed excellent antifungal activities against *A. niger*. All complexes were most sensitive to *A. flavus*. Moreover, The compounds H1, H5, H6, H7, H9 showed excellent antifungal activities. In conclusion, the Zn (II) complexes (H1, H5, H9) showed the highest antifungal activities against all fungal strains.

3.5.3. Minimum inhibitory concentration (MIC) for antimicrobial activity

All compounds showed variable average inhibitory activities against different strains ranging from 12.1 to 23. The data showed that compounds H1, H5 and H9 were the most active with average inhibition values of 21.9, 23 and 21.1 after preliminary antimicrobial screening. Therefore, these compounds were selected for MIC studies (Table 4). Their MIC values were in the range between 1.205×10^{-8} and 3.785×10^{-7} M, while H5 was proved to be the most active compound inhibiting the growth of *A. flavus* with 1.205×10^{-8} M.

4. Conclusions

In summary, we have developed a new solvent-free approach for the synthesis of a series of ferrocenyl chalcone Schiff bases and their metal (II) complexes. They were determined by several physicochemical and spectral analyses. Solvent-free method is cost-effective compared with liquid-phase method, which avoided the use of solvents, mild conditions, shorter time, higher yields and the catalyst could be recycled. The molar conductivity measurements indicated that all complexes were nonelectrolytes. Moreover, metal complexes had the more activities of antibacterial or antifungal than Schiff base ligands. The results of biological activity screening revealed that the Zn (II) complexes (H1, H5, H9) had significant activitis against all bacterial and fungal strains.

Acknowledgments

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