Synthesis, Characterization and in vitro Antibacterial Studies of 1, 3-Diones with their Metal Complexes bearing Potential O, Opharmacophores Sites

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ABSTRACT

This research communication is toward the investigation of the in vitroantibacterial activity of the synthesized compound ***4(L$_A$-L$_B$)*** *bearing potential O, O pharmacophores. These compounds have been obtained by the interaction of substituted 2-hydroxyacetophenones****1(A-B)*** *and aromatic acids****2(A-B)*** *underultrasound irradiation method at low temperature. The newly synthesized 1, 3-diketone****4(L$_A$-L$_B$)*** *andtheirmetal complexes* ***5(a-e)****were characterized by FT-IR, UV-Vis., ^{1}H-NMR, ^{13}C-NMR,Mass Spectroscopy and Magnetic measurement. Further these compounds showed potent antibacterial activity. A good correlation was obtained between the theoretical predictions of bioavailability and experimental verification. Utilization of ultrasound irradiation, simple reaction conditions, isolation and purification makes manipulation very important for economic and environmental approaches.*

KEYWORDS: *1,3-diketone,metal complexes, ultrasound irradiation,antibacterial screening.*

INTRODUCTION

The1,3-dioneshave broad spectrum of medicinal values which shown to have pharmacological activity like antibacterial [1], antiviral [2], insecticidal [3], antioxidant [4]and potential prophylactic antitumor activity [5-6]. It has also been used as in the anti-sunscreen agent [7]. In liquid solutions [8] as well as in the solid state [9],the1,3-diketone exists almost exclusively as the keto-enol tautomer, which is stabilized by the intermolecular hydrogen bonding. Recently it is reported that 1, 3-diketone are important pharmacophores of HIV-1 integrase (1N) inhibitors [10]. It was also reported that a number of diketones has warrant examination as breast cancer chemo preventive blocking agent [11] anticarcinogenic agent [12] and antistereogenicagent [13].

1,3-diketone and its metal complexes appear very promising for potential use as antibacterial agents due to their other biological properties [14-17].There is continues interest in synthesizing 1,3-diketone **4(L$_A$-L$_B$)**and its metal complexes**5(a-e)**because of their potential applications, applied sciences and importance area of coordination chemistry [18-21].In view of the above applications in the present paper we report the synthesis, characterization and antibacterial studies of metal complexes**5(a-e)**with 1,3-diketone **4(L$_A$-L$_B$).**

EXPERIMENTAL

Materials and methods

All chemicals used were of the analytical grade (AR) and of highest purity. Substituted 2-hydroxyacetophenone **1(A-B)** aromatic acid **2(A-B)** and dry pyridine were used for synthesis of ligand. AR grade metal nitrate used for complexes preparation. Spectroscopic grade solvent were used for spectral measurements. IR spectra in the range 4000-400cm^{-1} were recorded on Shimadzu FT-IR-4100 spectrometer using KBr pallets. ^{1}H-NMR and ^{13}C-NMR spectra of the ligand were recorded in $CDCl_3$ solvent. Mass spectra were taken on a Macro Mass spectrometer. The UV-Vis spectra of the complexes were recorded on Shimadzu UV-1800 Spectrophotometer. Magnetic measurements of the metal complexes were done on a Gouy balance at room temperature using Hg [Co (SCN) $_4$] as a calibrate.

Synthesis*of 2-acetylphenyl benzoate*3(A-B)

Equimolar amount of Substituted 2-hydroxyacetophenone **1(A-B)**and aromatic acid **2(A-B)**were dissolved in 15 mL dry pyridine. The reaction mixture was then cooled to 0 ^{0}C. To this, phosphorus oxychloride (0.06mol) was added drop wise maintaining temperature below 10^{0}C.Then the reaction mixture was irradiated for about 2-3h. It was then poured into 100 mL 1M HCl containing 50 gm crushed ice with vigorous stirring. The crimson colored solid was obtained which was filtered and washed several times with ice cold water. Compound **3(A-B)**was then recrystallized with distilled ethanol. Purity of the compound was checked by TLC.Thecompound **3(A-B)**was subjected to well known Baker-Venkatraman transformation.

*Synthesisof (Z)-1-(5-bromo-2-hydroxyphenyl)-3-(4-fluorophenyl)-3-hydroxy prop-2-en-1-one)*4(L_A)

A Compound containing **3(A)** 3.18 g, 0.01 mol)was dissolved in 15 mL dry pyridine. To this mixture, powdered KOH (1.12 g, 0.02 mol)was irradiated for about 1-2 h. Then it was poured over crushed ice and acidified with concentrated hydrochloric acid. The resulting solid **4(L_A)**was recrystallized from ethanol (Yield: 80%);m.p.:172^{0}C. Ana. Calcd (%) for $C_{15}H_{10}BrFO_3$(MW. =337.14g. mol^{-1}) C, 53.44; H, 2.99; Br, 23.70; F, 5.64; O, 14.24. IR (KBr) ν_{max}/cm^{-1}; 1744 (ν(C=O) ketonic), 1178 (ν (C-O) enolic), 3109 (ν (-OH) intramolecular H-bonding in Phenolic). ^{1}H-NMR (500 MHz, $CDCl_3$-d_6); δ /ppm = 15.56 (s, 1H, enolic -OH), 12.02 (s, 1H, Phenolic –OH), 7.55 (s, 1H, =C-H ethylene), 6.72-8.02 (m, 7H, Ar-H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ/ppm = 194.17 (C=O), 177.35 (C-O enolic), 91.85 (=C-H ethylene). UV/Vis. (DMSO) nm: 371, 256. MS *m/z*: 337.98.

Synthesis *of (Z)-3-hydroxy-1-(2-hydroxyphenyl)-3-(4-nitrophenyl) prop-2-en-1-one)* 4(L_B)

A Compound containing **3(B)**(3 g, 0.01mol)was dissolved in 15 mL dry pyridine. To this mixture, powdered KOH (1 g, 0.02 mol)was irradiated for about 1-2 h. Then it was poured over crushed ice and acidified with concentrated hydrochloric acid. The resulting solid **4(L_B)**was recrystallized from ethanol (Yield: 82%);m.p.:132 ^{0}C. Ana. Calcd (%) for $C_{15}H_{11}NO_5$(MW. =285.06 g. mol^{-1}) C, 63.16; H, 3.99; N, 4.91; O, 28.04. IR (KBr) ν_{max}/cm^{-1}; 1735 (ν (C=O) ketonic), 1199 (ν (C-O) enolic), 3099 (ν (-OH) intramolecular H-bonding in Phenolic). ^{1}H-NMR (500 MHz, $CDCl_3$-d_6); δ /ppm = 14.86 (s, 1H, enolic -OH), 11.87 (s, 1H, Phenolic –OH) 7.49 (s, 1H, =C-H ethylene), 6.54-7.98 (m, 8H, Ar-H); UV/Vis. (DMSO)nm:399,340.MS*m/z*:285.06

***Synthesis of metal complexes* 5(a-e)**

A hot ethanolic solution, 25 mL of ligand **(4L$_A$)**(0.002 M) and a hot ethanolic solution, 25 mL of required metal salt (0.001M) were irradiated for about 90 minute under ultrasound. Then a colored solid **5(a-e)**precipitate formed was filtered, washed with cold ethanol and dried under vacuum.All complexes were colored solids and air stable. (Yield: 82-87%) m. p. ≥300 ^{0}C.

Ana. Calcd. (%) for **5(a)** $C_{30}H_{22}Br_2F_2MnO_8$ (MW. = 763.23 g.mol^{-1}) C, 47.21; H, 2.91; Br, 20.94; F, 4.98; O, 16.77; Mn, 7.20; IR (KBr) ν $_{max}$/cm^{-1}; 1649 (ν (C=O) ketonic), 1143 (ν (C-O) enolic), 3072 (ν (-OH) intramolecular H-bonding in Phenolic), 3433 (ν (-OH) in H_2O molecules) 515 (ν (M-O bond in complex);UV/Vis. (DMSO) nm: 271 ($\pi \rightarrow \pi^*$),398 (LMCT); µeff (BM):5.86

Ana. Calcd. (%) for **5(b)** $C_{30}H_{22}Br_2F_2FeO_8$ (MW. = 764.14 g. mol-^{1}) C, 47.15; H, 2.90; Br, 20.91; F, 4.97; O, 16.75. Fe, 7.31; IR (KBr) ν $_{max}$/cm^{-1}; 1647 (ν (C=O) ketonic), 1145 (ν (C-O) enolic), 3070 (ν (-OH) intramolecular H-bonding in Phenolic), 3367 (ν (-OH) in H_2O molecules), 526 (ν (M-O bond in complex); UV/Vis. (DMSO) nm:272 ($\pi \rightarrow \pi^*$), 397 (LMCT), 672 (d-d); µeff (BM):6.33

Ana. Calcd. (%) for **5(c)** $C_{30}H_{22}Br_2F_2CoO_8$ (MW. = 767.23 g. mol-^{1}) C, 46.96; H, 2.89; Br, 20.83; F, 4.95; O, 16.68. Co, 7.68; IR (KBr) ν $_{max}$/cm^{-1}; 1645 (ν (C=O) ketonic), 1147 (ν (C-O) enolic), 3070 (ν (-OH) intramolecular H-bonding in Phenolic), 3464 (ν (-OH) in H_2O molecules), 522 (ν (M-O bond in complex); UV/Vis. (DMSO) nm:270 ($\pi \rightarrow \pi^*$), 392 (LMCT), 674 (d-d); µeff (BM):4.26

Ana. Calcd. (%) for **5(d)** $C_{30}H_{22}Br_2F_2NiO_8$ (MW. = 766.99 g. mol-^{1}) C, 46.98; H, 2.89; Br, 20.84; F, 4.95; O, 16.69. Ni, 7.65; IR (KBr) ν $_{max}$/cm^{-1}; 1645 (ν (C=O) ketonic), 1143 (ν (C-O) enolic), 3072 (ν (-OH) intramolecular H-bonding in Phenolic), 3450 (ν (-OH) in H_2O molecules), 526 (ν (M-O bond in complex); UV/Vis. (DMSO) nm:271 ($\pi \rightarrow \pi^*$), 401 (LMCT), 674 (d-d); µeff (BM):2.50

Ana. Calcd. (%) for **5(e)** $C_{30}H_{22}Br_2F_2CuO_8$ (MW. = 771.84 g. mol-^{1}) C, 46.68; H, 2.87; Br, 20.70; F, 4.92; O, 16.58. Cu, 8.23; IR (KBr) ν $_{max}$/cm^{-1}; 1648 (ν (C=O) ketonic), 1149 (ν (C-O) enolic), 3030 (ν (-OH) intramolecular H-bonding in Phenolic), 3426 (ν (-OH) in H_2O molecules), 513 (ν (M-O bond in complex); UV/Vis. (DMSO) nm:276 ($\pi \rightarrow \pi^*$), 393 (LMCT), 673 (d-d); µeff (BM):2.1

RESULTS AND DISCUSSION

The elemental analysis show 1:2 (metal: ligand) stoichiometry for all the complexes. The structure of the compounds characterized by spectral analysis. The magnetic measurement studies showed that the complexes **5(a-e)** have octahedral geometry [22]. All complexes showed higher antibacterial activity than the free ligands.

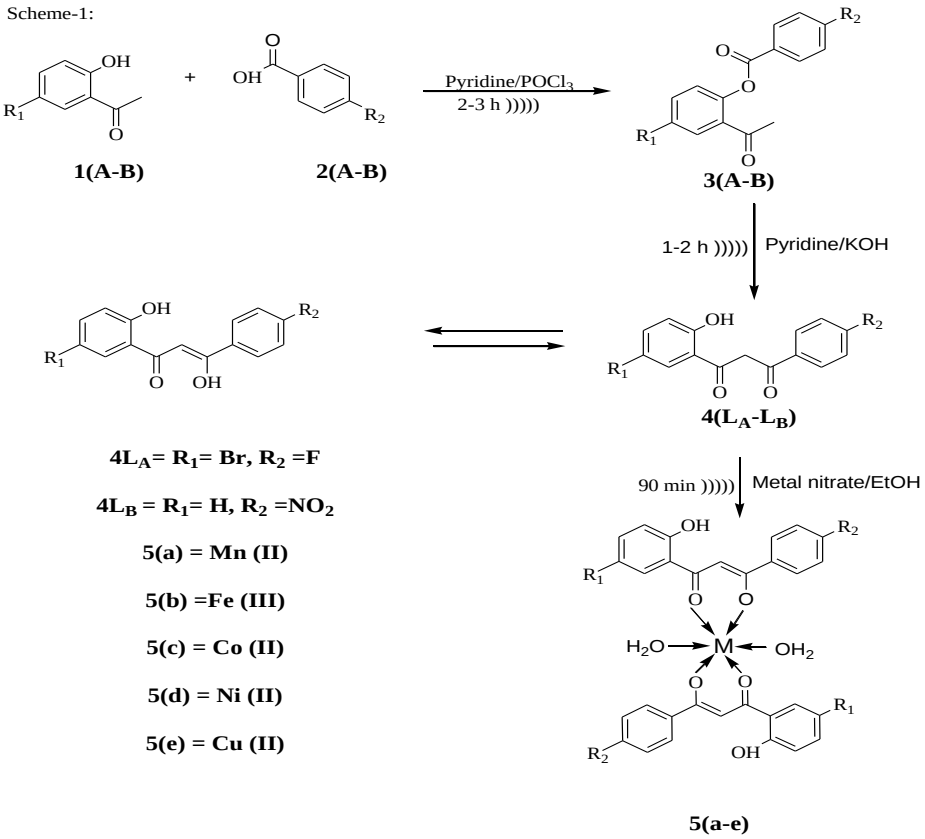
Conventional and ultrasound irradiation techniques

Table 1. Physical characterization and ultrasonic study of ligand and its metal complexes.

Ligand/Complexes	Mol.Wt.	M.P./decomp. Temp (^{0}C)	Conventional		Ultrasound Irradiation [a]	
			Time	Yield (%)	Time	Yield (%)
4L$_A$	337.14	172	370	68	120	80
4LB	285.25	132	370	70	120	82
5a	763.23	≥300	280	72	90	82
5b	764.14	≥300	280	68	90	80
5c	767.23	≥300	280	70	90	85

5d	766.99	≥300	280	73	90	84
5e	771.84	≥300	280	74	90	87

Synthesis of ligands and its metal complexes



Magnetic measurements

The magnetic measurements of complexes were measured at room temperature. The observed magnetic moment value of **(5a)** complex is 5.86 BM, **(5b)** complex is 6.33 BM, **(5c)**complex is 4.26 BM, **(5d)** complex is 2.50 BM, and **(5e)** complex is 2.12 BM. The magnetic measurement studies showed that the all complexes have octahedral geometry [23-26].

Spectroscopic analysis

The ^{1}H-NMR spectrum of the compound **4(L_A-L_B)** exhibited a singlet at δ 15.56 and 14.80 ppm due to enolic proton a singlet at δ 12.02 and11.87 ppm due to phenolic proton adjacent to the carbonyl group and a singlet at δ 7.55 and 7.49 ppm respectively showed ethylene proton indicate that keto- enol form in 1,3-diketone is more stable. The ^{13}C NMR spectrum gives a singlet at δ 194.17 ppm due to ketonic carbon and δ 177.35 ppm due to enolic carbon, confirming the keto-enol tautomer in 1, 3-diketone. The characteristics infrared spectral assignment of ligand **4(L_A-L_B)** and their metal complexes **5(a-e)** the presence of broad band at 3109 and 3099cm^{-1} exhibited intramolecular hydrogen bonding due to –OH group. The spectra of ligand assigned to carbonyl group (C=O) and 1178cm^{-1} due to (C-O)

stretching, exhibited a lower shift of 10-20 cm^{-1} in metal complexes **5(a-e)**.This shift indicates that the keto-enol tautomer in ligand **4(L_A-L_B)** coordinated with the transition metal ion. All the above evidences were further supported by the emergence of new bands at 515-526 cm^{-1} due to metal-oxygen vibrations. These new bands observed in the spectra of the transition metal complexes only.

Antibacterial activity

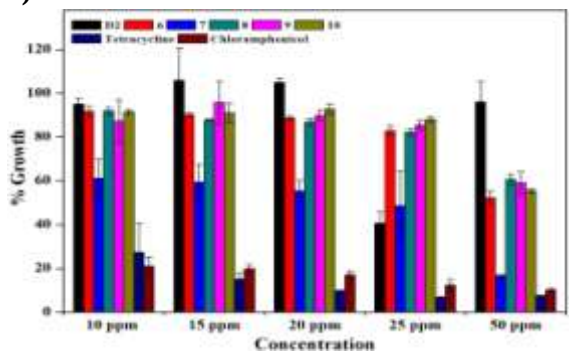
Two Gram-positive (*B. subtilis* NCIM 2063 and *S. aureus* NCIM 2079) and two Gram-negative (*E. coli* NICM 2065 and *P. aeruginosa* NCIM 2200) bacteria were used as test organisms. The positive control drug tetracycline and chloramphenicol were dissolved in DMSO at a concentration of 1 mg/ml and further diluted (1:10) in Milli Q water. The two fold dilution of the compounds and reference drugs were prepared (10, 15, 20, 25 and 50 ppm). Antibacterial activities of the bacterial strains were carried out in nutrient broth with inoculums of 10^3 cells ml^{-1} by the spectroscopic method and an aliquot of 30 µl was added to each tube of the serial dilution. The chemical compounds were added in nutrient broth medium with bacterium and incubated on a rotary shaker at 37 °C for 24 h at 150 rpm. The percentage growth was calculated by the following equation [27].

$$\% \text{ Growth} = (\text{OD at } 600\text{nm Sample})/(\text{OD at } 600\text{nm control}) \times 100$$

Table 2. MIC of ligand and its metal complexes for antibacterial activity

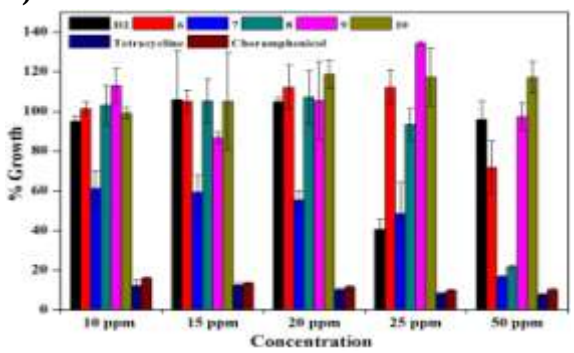
Samples	***E.coli*(NCIM 2065) (ppm)**	***B. subtilis*(NCIM 2063) (ppm)**	***P. aeruginosa*(NCIM 2200)(ppm)**	***S. aureus*(NCIM 2079) ppm)**
$4L_A$	25	25	>50	50
5a	>50	50	>50	50
5b	>50	>50	>50	50
5c	>50	>50	>50	50
5d	25	25	20	50
5e	50	>50	>50	50
Tetracycline	< 10	< 10	< 10	< 10
Chloramphenicol	< 10	< 10	< 10	< 10

a)

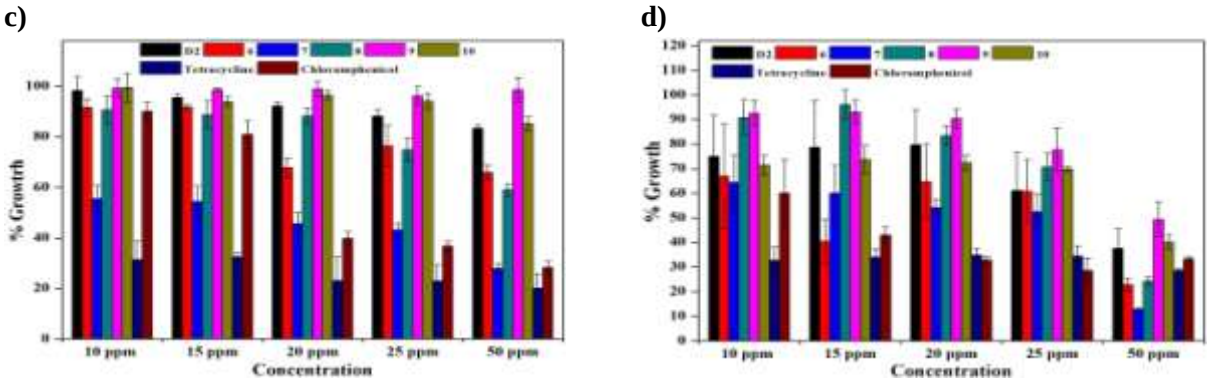


E.coli

b)



B. subtilis



P. aeruginosa ***S. aureus***

Figure 1. Antibacterial screening: percentage growth of tested samples.

Compound **(4L$_A$)** showed maximum growth inhibitory effect on *E. coli* and *B. subtilis* as compared to *S. aureus* and *P. aeruginosa* at 25ppm concentration. Amongst the Samples **(5a)-(5e)** only sample **(5d)** exhibited the growth inhibitory action on *E.coli*, *B.subtilis and P. aeruginosa* at lower concentration of 20 ppm. Remaining all samples did not showed any growth reduction of other bacteria except *S. aureus* at 50 ppm concentration. The Ni (II) complex **(5d)** has found to be more promising compared to other metal complexes. From the table (2) and fig. (1), it is clear that the results are in good agreement with respect to activity of free ligand and its metal complexes [28-29].

CONCLUSIONS

It has been suggested that the antibacterial activity of the ligand **(4L$_A$)**increased upon coordination with the transition metal complexes **(5a)-(5e).**The chelating process reduces the polarity of metal ion by coordinating with ligand, which increases the lipophilic nature of the metals. This lipophilic nature of the metal enhanced its penetration through the lipoid layer of cell membrane of the microorganism. The synthesized ligand **4(L$_A$-L$_B$)** having O, O pharmacophores site played an important role to increased antibacterial activity. Thus it is concluded that the compounds were found to possess a broad range of hydrophilic and lipophilic characters, hence indication of favorable bioavailability based on drug likeness.

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