Synthesis, Characterization and Antimicrobial Activity of 2-[1-(2,4-Dihydroxyphenyl) Ethylene]Hydrazine Carboxamide and its Zn(II) Metal Complex

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Abstract. The synthesis of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide and its Zn(II) metal complex was achieved. 2, 4 dihydroxy acetophenone is synthesized by resorcinol with glacial acetic acid. This further treated with semicarbazide hydrochloride to form its semicarbazone. Zn(II) metal complex was prepared by dissolving equimolar quantities of metal salt and Schiff base ligand in ethanol. All the target compounds were characterized by M.P, TLC and UV-visible and IR spectral data. Antimicrobial activities of these synthesized compounds were studied in sterile saline by Agar well diffusion method against *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* bacteria. All the synthesized compounds have shown good to moderate antimicrobial activity.

Introduction

Hydroxy acetophenones and its derivatives were used as starting material for the synthesis of chalcones [1], flavones [2] and Schiff bases [3, 4] etc. Hydroxy acetophenone semicarbazone have wide spectrum of biological activities. Schiff bases and their co-ordination compounds have great importance as they are useful in biochemical [5], anticancer [6], anti-inflammatory [7] and antipyretic [8]. A Schiff base of hydroxyl acetophenone and its complexes has variety of applications in biological, clinical, analytical and pharmaceutical area [9, 10]. Metal ions are the factors in the structural organization of biochemical molecules and the functional processes operating in the genetic and metabolic systems [11]. Transition metal ions and their complexes are the essential components found in all living organisms [12]. The interaction of transition metal ions with biologically active ligands is a subject of interest. Studying the biological activities of metal complexes is interesting. Thus the aim of the present work is to synthesize some new hydroxyacetophenones, its derivatives, Zn metal complex and to study their antimicrobial activities [13-18]. Their structures were elucidated on the basis of MP, TLC, elemental analysis, UV-visible and IR spectral data. All the synthesized compounds have been screened for their biological activity by Agar well diffusion method.

Materials and Methods

The chemicals used in the present work were dry resorcinol, glacial acetic acid, powdered Zinc chloride, semicarbazide hydrochloride, anhydrous sodium acetate, ethanol etc. all these chemicals were purchased from Zen Scientific, Mumbai and were of AR grade. All these chemicals were used for synthesis.

Test Microorganism: The microorganism used in the present study were *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*. Microorganisms were maintained at 4°C on nutrient agar slants.

The reagent 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide and its Zn(II) metal complex was prepared as reported in the literature. The structure of the Schiff base is confirmed by using physical methods like melting point, TLC, elemental analysis, UV-visible and IR spectra. The newly synthesized Zn complex was characterized on the basis of microanalysis data, elemental analysis, UV-visible and IR.

Synthesis of 2,4-dihydroxyacetophenone

Briefly, the synthesis was carried out by dissolving freshly fused and powdered zinc chloride (0.24 mole) in 32 ml of glacial acetic acid by heating on sand bath. Dry resorcinol (0.2 mole) was added with stirring at 140°C. The solution was heated until it just begins to boil and kept for 20 minutes at 150°C. Dilute HCl (1:1) was added to the mixture and the solution was cooled to 5°C. The separated product was filtered and washed with dilute HCl. The product was recrystallized from hot water. The reaction Scheme is given in Fig. 1.

Reaction Scheme:

Figure 1. Reaction scheme for the synthesis of 2,4-dihydroxyacetophenone

Synthesis of Semicarbazone Derivative:

1 gram of powdered semicarbazide hydrochloride was added 0.9 gram of anhydrous sodium acetate to 5ml of water and was warmed gently until a clear solution is appeared. A solution of 1gm of 2, 4 dihydroxyacetophenone in 5ml of ethanol was added. The mixture was warmed gently in a water bath for 15 mins. The semicarbazone rapidly crystallizes. Finally, cool filter off the 2,4 dihydroxyacetophenone Semicarbazone, washed thoroughly with water and was drained. Recrystallised from ethanol. Melting point is 201°C. The reaction Scheme is given in Fig. 2.

Reaction Scheme:

Figure 2. Reaction scheme for the synthesis of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Synthesis of Zn(II) Complex:

The ligand (Schiff base) (0.1mm) was taken in hot ethanol (40mL) and treated with a hot ethanolic solution of ZnCl_2 (0.1mm) the light yellow transparent solution of ligand changed to colourless after mixing the solution. This reaction mixture was refluxed for 8 hrs and mixture of solution was cooled, left to stand overnight, filtered by under suction. Crystalline solid was separated out and recrystallized from ethanol. The yield 70% and melting point 345°C . The reaction Scheme is given in Fig. 3.

Reaction:

Figure 3. Reaction scheme for the synthesis of Zn Complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Spectral Analysis: The structure of all synthesized compounds was confirmed by Elemental analysis, Uv-visible and IR Spectroscopic technique. The absorbance measurement was carried out on Shimadzu Uv-visible 2100 spectrophotometer with 1 cm quartz cell. The IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method.

Figure 4. IR for 2,4- dihydroxyacetophenone

Figure 5. IR for 2,4- dihydroxyacetophenone Semicarbazone

Figure 6. IR for Zn Complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Antimicrobial studies: For antimicrobial activity the suspension of synthesized compounds and Zn(II) metal complex was made with sterile saline. In the present work the antimicrobial activities of the synthesized compounds have been screened against *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*.

Preparation of test compounds: The solutions were prepared at a concentration of 1mg/μl for all the compounds.

Preparation of Plates and Microbiological assay: The antimicrobial evaluation was done by Agar well diffusion method [19, 20] using Mueller Hinton Agar no.2 as the nutrient medium. The bacterial strains were activated by inoculating loop full of strain in 20 cm³ of nutrient agar and the same was incubated for 36 hrs. in an incubator at 37°C. 0.1 cm³ of the activated strain was inoculated in Mueller Hinton agar. Mueller Hinton agar was kept at 45°C and then poured in the

petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 36 hrs. at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of these synthesized compounds.

Results

2,4- dihydroxyacetophenone, its semicarbazone derivative and its Zn(II) metal complex are synthesized. The synthesized compounds were purified by recrystallization method. The purity of the compounds was checked by TLC using appropriate solvent systems. The physical and analytical parameters were checked. (Table1) The structure of all the synthesized compounds was confirmed by UV-visible and IR spectroscopic technique.

Spectral Data

2,4-dihydroxyacetophenone: IR(cm^{-1} , KBr) 3307(aro, O-H str), 3197.20(aro, C-H str), 1521.79(aro, C=C str), 1925.97(OH para sub), 1800(OH ortho sub), 1617.30(C=O conj), 1143.96(aro, C-O str), 823.30(para sub), 729.16(ortho sub). (Table 2)

2,4-dihydroxyacetophenone semicarbazone: : IR(cm^{-1} , KBr) 3309(aro, O-H str), 3212.72(aro, C-H str), 1525.44(aro, C=C str), 1213.08(C-N str), 1143.83(aro, C-O str), 1688.10(C=O conj), 1143.96(aro, C-O str), 839.28(para sub), 728.93(ortho sub). (Table 2)

Zn Complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide: IR(cm^{-1} , KBr) 3167.96(aro, O-H str), 1586.21(aro, C=C str), 1283.70(C-N str), 1156.36(aro, C-O str), 1681.61(sec/tert amide), 836.67(para sub), 512.97 (M-O str) (Table 2)

Antimicrobial Activity

Microbial assay was carried out by agar well diffusion method. The bacterial strains were activated by inoculating loop full of strain in 20 cm³ of nutrient agar and the same was incubated for 36 hrs. in an incubator at 37°C. The zone of inhibition in different bacterial strains i.e. *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* against synthesized compounds shown in Table 3. Among the various bacterial strains maximum zone of inhibition was recorded in *S. aureus* and minimum zone of inhibition was observed in *M. luteus* and *P. aeruginosa*.

Table 1. Physical and analytical data

Compound	Color	M.P.(°C)	λ_{max} in nm	Elemental analysis%		
				C	H	N
I	Off white	143	217	63.15	5.30	--
II	Pale yellow	201	342	59.01	3.90	5.45
III	Yellow	345	385	58.40	3.43	6.62

Table 2. Spectral Data

Group & vibration	Frequency's in cm^{-1} I	Frequency's in cm^{-1} II	Frequency's in cm^{-1} III
Aro, O-H Streching vibration	3307	3309	3167.96
Aro, C-H Streching vibration	3197.20	3212.72	-----
Aro, C=C Streching vibration	1521.79	1525.44	1586.21
OH para subsituted	1925.97	1922	-----
OH ortho subsituted	1800	1805	-----
C=O conjugation	1617.30	1688.10	-----
Aro, C-O Streching vibration	1143.96	1143.83	1156.36
Para substitution	823.30	839.28	836.67
Ortho substitution	729.16	728.93	727
Aro, C-N streching	----	1213.08	1283.70
Tertiary amide	----	----	1681.61
M-O streching	----	----	512.97

Table 3. Antibacterial activity

Entry	Substt	Zone of Inhibition in mm				
		Gram positive Bacteria			Gram negative Bacteria	
		<i>M. luteus</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1.	I	12	15	19	13	12
2.	II	14	16	21	15	13
3.	III	15	18	25	23	15
4.	Ampicillin	17	19	26	24	17

I 2,4- dihydroxyacetophenone

II 2,4- dihydroxyacetophenone Semicarbazone

III Zn Complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Discussion

The results indicate that inhibition depends on strain and structure. In compound I due to presence of C=O only it shows less inhibition. But in compound II due to presence of C=N imine group inhibition increases comparison to compound I. Also a broad band at 3500-3400 is attributed to intermolecular hydrogen bonded -OH group, at 1600-1700 C=O (amide) strong absorption, Medium sharp absorption band around 3200-3100 N-H stretching vibration, Strong absorption band 1600-1675 due to tertiary amide. Aromatic C-H band at 3000-2900. Physicochemical studies have been used in the elucidation of the geometry of the metal complexes with the purpose of throwing light on the structural aspects of newly synthesized Schiff base complexes. The newly synthesized ligand and its Zn(II) metal complex were characterized by elemental analysis, UV-visible, IR, TLC and repeated MPs determination studies. The susceptibility of the contain strains of bacteria towards the synthesized compounds was determined by measuring the size of inhibition diameter. Zn(II) complexes were highly effective against *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* than their parent compound. It was also observed that antibacterial activity of these complexes for removing bacteria was fairly good.

Conclusions

2,4- dihydroxy acetophenone, its semicarbazone derivative and Zn(II) metal complex were synthesized. The structures of synthesized compound were supported by IR spectroscopic technique. The antibacterial screening of these compounds shows that inhibition depends on strain

and structure. Presently, there is an emergence of multiple drug resistance to human pathogenic organism. So there is an urgent need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The clinical isolated bacteria in this study were *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*. These isolates of bacteria caused high percentage of drug resistance. The antibacterial study showed that complexes are found to be more active against *M. luteus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*. Compared to standard antibacterial compound the complexes show moderate activity against the selected strains of microorganisms.

Conflict of Interest

The author declares that there is no conflict of interest.

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