



INVESTIGATION OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF NEWLY SYNTHESIZED SAMARIUM(III) COMPLEX WITH B-HYDROXYKETONE AND 2,2'-BIPYRIDINE AS ANCILLARY LIGAND

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Article Received on 28/12/2017

Article Revised on 18/01/2018

Article Accepted on 08/02/2018

ABSTRACT

New ternary Sm (III) complex, Sm (CHME) 3.bipy was synthesized by adopting solution precipitation method by employing β -hydroxyketone ligand 2-(4-chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl) ethan-1-one (CHME) as main ligand and 2,2'-bipyridine (bipy) as ancillary ligand. The structure of ligand and complex were confirmed by various techniques i.e. elemental analysis, FT-IR spectroscopy, ¹H-NMR spectroscopy. The synthesized ligand and their corresponding complex were screened for their in vitro antimicrobial and antioxidant activities by tube dilution and DPPH method respectively. The complex was found to be promising candidate as new antimicrobial agent.

KEYWORDS: Sm (III) complex, β -hydroxyketone, 2,2'-bipyridine, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

The broad use of antibiotics has led to the development of bacterial resistant microbial pathogens. They either inhibit the growth of microorganism or kill them. The mounting incidence of infection caused by rapid development of bacterial resistance to most of the known antibiotics is a serious health problem.^[1-4] Firstly, bacteria and fungi generally develop metabolizing enzymes for the degradation of the drugs, modifying their targets to nil the effective of the drugs and expressing a high level of efflux protein that pump the drug out in order to lower its concentration.^[5-10]

The antimicrobials have been used in common practice from ancient times to treat infections caused by microorganisms. Globally a lot of progress has been done to design and develop new antimicrobial agents to combat the frightful incidences of re-emerging infectious diseases. But still, the statistics of incidents of havoc by these microorganisms are really alarming. Therefore, this area of research also needs special attention consequently for the synthesis of novel biological active compounds as well as the alternation of known drugs to minimize their resistance to pathogens having same physiological action and targeting unique mechanisms and pathways.^[11]

The implications of oxidative damage in various pathological events like as cancer and aging is induced by free radicals and reactive oxygen species.

Antioxidants are the compounds that prevent such oxidative damage due to their free radical scavenging ability.^[12] In β -hydroxyketone ligand, such ability is attributed to phenolic-OH group is presented in it. Therefore, the main goal of the present research is to evaluate in vitro antimicrobial and antioxidant activities of newly synthesized β -hydroxyketone and their corresponding Sm (III) complex.

EXPERIMENTAL

METHODS AND MATERIALS

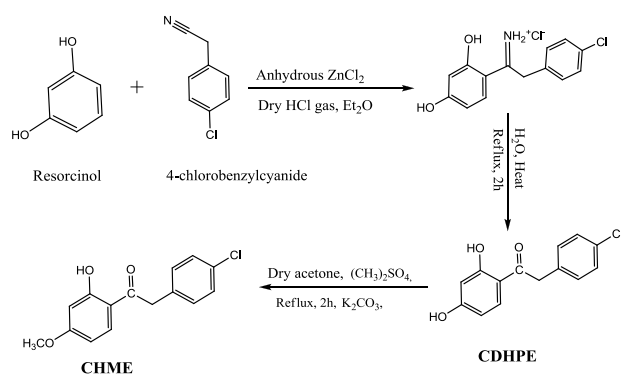
Sm (NO₃)₃.6H₂O and 2,2'-bipyridine were purchased from Sigma-Aldrich. Resorcinol, 4-chlorobenzylcyanide, anhydrous zinc chloride, potassium carbonate, dimethyl sulphate were purchased from Hi-Media Pvt Ltd. The reagent employed were of analytical grade and used without any further purification. All Microbial strains *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Escherichia coli* (MTCC 443) and fungal strains: *Candida albicans* (MTCC 227) *Aspergillus niger* (MTCC 281) were purchased from Institute of microbial Technology, Sector 39-A, Chandigarh, India. Nutrient broth medium, nutrient agar medium, subourand dextrose agar medium and subourand dextrose broth medium, dimethyl sulfoxide (DMSO) were purchased from Hi-Media Pvt Ltd. The characterization of synthesized ligand CHME and its corresponding Sm(III) complex have been done by various techniques. Elemental analysis was performed

using thermo scientific flash 2000 elemental analyzer, while the samarium content of the complex was estimated by complexometric titration with EDTA by using xylenol orange as an indicator. FT-IR spectra were obtained on Perkin Elmer spectrum 400 FT-IR spectrometer by using KBr disks in the range of 4000-400 cm^{-1} . The nuclear magnetic resonance spectra were recorded on Bruker Avance II 400 spectrometer (400 MHz) in dimethylsulfoxide solution. The antimicrobial and antioxidant activities were investigated of the synthesized ligand and Sm (III) complex by tube dilution method and DPPH method respectively.

Synthesis

Synthesis of ligand 2-(4-chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl) ethan-1-one (CHME)

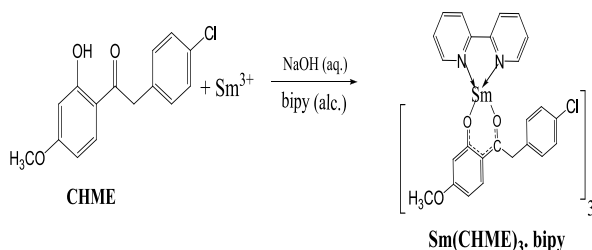
The ligand CHME was synthesized by conventional method as per literature^[13] and is given in scheme 1 as follows:



Scheme 1: The synthetic route of ligand CHME.

Synthesis of complex $\text{Sm}(\text{CHME})_3\text{bipy}$

$\text{Sm}(\text{CHME})_3\text{bipy}$ complex was synthesized by adding 3 mmol CHME and 1 mmol of 2,2'-bipyridine dissolved in ethanol to an aqueous solution of 1 mmol $\text{Sm}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ with constant stirring on magnetic stirrer. The pH value of the mixture was adjusted to 7-8 by adding 0.05 M NaOH aqueous solution. The mixture was stirred for 3h at 35 °C and then allowed to stand for 1 h. White precipitates appeared in the reaction mixture which were purified by washing with deionized water and then with ethanol to remove the free ligand. $\text{Sm}(\text{CHME})_3\text{bipy}$ complex was white powder with 80 % yield.



Scheme 2: The synthetic route and structure of $\text{Sm}(\text{CHME})_3\text{bipy}$ complex with CHME.

Biological evaluation

Antimicrobial Activity

The ligand and the synthesized complex were evaluated for their *in vitro* antimicrobial activity against the Gram-positive bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and Gram-negative bacterium: *Escherichia coli* (MTCC 443) and fungal strains: *Candida albicans* (MTCC 227) *Aspergillus niger* (MTCC 281) using conventional tube dilution method.^[14] Ciprofloxacin and fluconazole were the standard drugs for antibacterial and antifungal testing respectively.^[15,16] For bacteria, dilutions were prepared in double strength nutrient broth I.P and for fungi Sabouraud dextrose broth I.P.^[17] by using DMSO to give final concentration of 100 $\mu\text{g}/\text{mL}$. The incubation period for ligand and synthesized complex were 24 h at 37 °C for (bacteria), 48 h at 37 °C for (*Candida albicans*) and 7 days at 25 °C for (*Aspergillus niger*) respectively and the observed data were recorded in terms of MIC (the lowest concentration of compound required for a complete inhibition of inoculated bacteria and fungi after inocubation time).

Antioxidant activity

Nitrogen centered most familiarly used stable free radical 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH), was most employed to determine antioxidant activity spectrophotometrically based on the fact that odd electron present in the DPPH free radical reacts with antioxidant complex, its deep violet color in methanol bleaches to yellow, gives a strong absorbance, which maximises at $\lambda = 517\text{nm}$.^[18] Stock solution of various concentrations (25, 50, 75 and 100) $\mu\text{g}/\text{ml}$ of the complex dissolved in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. The mixtures were vigorously shaken and allowed to stand for 30 minutes in the dark at room temperature for incubation. Tests were carried out in triplicate. By using UV spectrophotometer, absorbance value was measured at $\lambda = 517\text{ nm}$. The DPPH scavenging activity was expressed as IC_{50} , whose concentration is sufficient to obtain 50% of maximum scavenging activity. Standard curve was plotted for different concentration of ascorbic acid and complex. Scavenging activity of DPPH free radical was calculated as:

$$\text{DPPH scavenging activity (\%)} = 100 \frac{(A_0 - A_s)}{A_0}$$

Where, A_0 is the absorbance of the control reaction (containing all reagents except test complex) and A_s is the absorbance of the test complex. The observed results were compared with the activity of ascorbic acid, which was used as the standard.

RESULTS AND DISCUSSION

Solubility

CHME and $\text{Sm}(\text{CHME})_3\text{bipy}$ were stable under atmospheric condition. The solubility investigated in various solvents. The Sm(III) complex were soluble in DMSO, DMF, chloroform, dichloromethane and acetone,

sparingly soluble in methanol and ethanol but insoluble in hexane, benzene and dichloromethane.

Elemental analysis, ¹H-NMR and IR Spectra

The elemental analysis data for CHME (C₁₅H₁₃O₃Cl) was found (calc.) % C, 65.18 (65.10); H, 4.76 (4.73); O, 17.29 (17.34). IR (KBr) cm⁻¹: 3429 (b), 3055 (m), 3022 (w), 2940 (w), 2842 (w), 1650 (s), 1633 (s), 1596 (s), 1567 (s), 1494 (s), 1439 (s), 1351 (s), 1310 (m), 1291 (s), 1229 (s), 1207 (s), 1197 (s), 1084 (s), 1028 (s), 994 (s), 822 (s), 810 (s), 777 (s), 600 (s), 581 (s). ¹H-NMR (400MHz, DMSO): δ 3.83 (s, 3H, OCH₃), 4.18 (s, 2H, CH₂), 6.42 (s, 1H, Ar-H), 6.43 (s, 1H, Ar-H), 7.24 (d, 2H, Ar-H), 7.30 (d, 2H, Ar-H), 7.57 (s, 1H, Ar-H), 12.61 (s, 1H, OH).

The elemental analysis data for Sm(CHME)₃bipy (C₅₅H₄₄O₉Cl₃N₂Sm) was found (calc.) % C, 58.32 (58.27); H, 3.94 (3.91); O, 12.76 (12.70); N, 2.52 (2.47); Sm, 13.34 (13.26). IR (KBr) cm⁻¹: 3072 (w), 2938 (m), 1890 (w), 1626 (s), 1573 (s), 1521 (s), 1420 (s), 1360 (s), 1242 (s), 1221 (s), 1133 (s), 1092 (s), 1012 (m), 977 (m), 843 (m), 812 (m), 802 (m), 586 (m), 484 (m). ¹H-NMR (400MHz, DMSO): δ 3.78 (s, 9H, OCH₃), 4.22 (s, 6H, CH₂), 6.50 (bd, 6H, Ar-H), 7.32 (b, 14H, 12Ar-H, 2bipy), 8.21 (s, 3H, Ar-H), 8.48 (d, 2H, bipy), 8.82 (d, 2H, bipy), 9.34 (d, 2H, bipy).

The structure of the synthesized ligand and its corresponding Sm(III) complex are given in Scheme 1 and 2 was confirmed by the elemental analysis. The above elemental analytical data indicate the stoichiometry of the ternary complex Sm(CHME)₃bipy to be 3:1:1 (CHME: Sm: bipy).

¹H-NMR spectra of the ligand CHME shows a singlet at δ 13.84 due to phenolic proton. This proton signal of OH band disappeared in the spectra of Sm(III) complex, indicating that oxygen atom of the phenolic OH coordinated with the Sm(III) ion. In the IR spectra of the ligand CHME (as tabulated in Table 1) a broad band appeared in the range 3500-3200 cm⁻¹ centred at 3429 cm⁻¹, which was a typical peak of free OH stretching vibration, which was absent in the ternary complex, indicating that ligand was coordinated with Sm(III) ion through oxygen atom of phenolic group. Further, an intense band at 1633 cm⁻¹, which corresponds to the C=O stretching vibration band of carbonyl group is present in the ligand. After coordination the C=O stretching frequency of the complex were red shifted to 7 cm⁻¹ indicating that oxygen atoms in the carbonyl group were also coordinated with Sm(III) ion.^[19]

Table 1: The characteristic IR bands (cm⁻¹) of the free ligand CHME and its corresponding Sm(III) complex.

Compound	ν(O-H)	ν(C=O)	ν(C=N)	ν(C=C)	ν(Ph-O)	ν(Sm-N)	ν(Sm-O)
CHME	3429	1633	---	1567	1229	---	---
Sm(CHME) ₃ bipy	---	1626	1573	1521	1221	586	484

A new absorption band appeared at 1573 cm⁻¹ in the spectrum of complex, assigned to ν(C=N), indicating that nitrogen atom of the ancillary ligand participated in the coordination with the central metal ion. It was further confirmed by the presence of medium intensity band at 586 cm⁻¹ attributed to the ν(Sm-N) band due to introduction of ancillary ligand.^[20, 21] The appearance of ν(Sm-O) band at 484 cm⁻¹ supports the chelation through the oxygen atom of the free ligand.^[22,23] Overall, the result of ¹H-NMR and infrared spectra indicated that the free ligand CHME was coordinated with Sm(III) ion through the phenolic hydroxyl group, carbonyl group and nitrogen atoms of the ancillary ligand 2,2'-bipyridine.

Antimicrobial activity

The in vitro antimicrobial activity activity of the synthesized novel Sm (III) complex was investigated against Gram-negative bacterium: *E. coli* (MTCC 443),

Gram-positive bacteria: *B. subtilis* (MTCC 441), *S. aureus* (MTCC 3160) and fungal strains: *C. albicans* (MTCC 227) and *A. niger* (MTCC 281). Tube dilution method was adopted for screening these antimicrobial strains. Inhibition zone were measured and recorded in Table 2, whereas the bar diagram Fig.1 represented the antimicrobial activity of Sm(III) complex with respect to standard drugs ciprofloxacin and fluconazole. The ligand was found to be inactive against all the investigated bacterial and fungal strains whereas Sm(CHME)₃bipy showed excellent activity compared to the standard antibiotics. In addition, it was interesting to note that Sm(CHME)₃bipy proved to be better than the standard ciprofloxacin against *S. aureus*. The increase in antimicrobial activity from ligand to complex may be due to the presence of Sm (III) ion coordinated with the donor atom of the ligand which leads to the π- electron delocalization.

Table 2: Minimum inhibitory concentration of CHME and its corresponding Sm(III) complex. The bold values indicate highest values of the respective properties.

Compounds	Minimum Inhibitory Concentration (μM/mL)				
	<i>B. subtilis</i>	<i>S.aureus</i>	<i>E.coli</i>	<i>C.albicans</i>	<i>A.niger</i>
CHME	22.5	22.5	22.5	45.2	45.2
Sm(CHME) ₃ bipy	11.0	5.51	11.0	11.0	11.0
Std.	8.71 ^a	8.71 ^a	8.71 ^a	10.09 ^b	10.09 ^b

^aCiprofloxacin ^bFluconazole

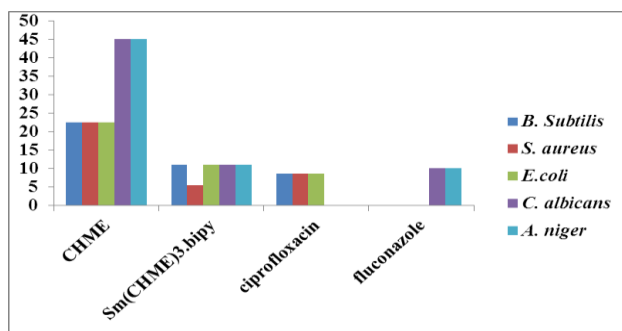


Fig. 1: Bar diagram of Sm (III) complex Sm (CHME)₃. bipy with ligand CHME showing the antimicrobial activities with respect to standard drugs.

v) Antioxidant activity

The antioxidant activity of the synthesized ligand CHME and their corresponding Sm(III) complex were evaluated by DPPH radical scavenging assay.^[24-28] It is due to fact

that odd electron present in the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) reacts with the antioxidant complex, which can donate hydrogen, it gets reduced and its deep violet color in methanol bleaches to yellow, shows a significant absorption decrease at 517 nm. Stock solution of various concentrations is 25, 50, 75 and 100 µg/mL of the test ligands and complex, dissolved in methanol were added to 5 mL of 0.004% methanol solution of DPPH. Then the mixture was shaken and allowed to stand for 30 min in the dark at room temperature for incubation. The IC₅₀ was calculated and summarized in Table 3 and graphically represented in Fig.2. The IC₅₀ values of ligand (IC₅₀= 64.84µg/ml) as well as all the Sm(III) complex (IC₅₀= 61.77µg/ml) were comparatively higher than standard ascorbic acid (IC₅₀= 43.78µg/ml) resulted that the ligand and its respective Sm(III) complex were found to be less effective antioxidant.

Table 3: Percentage inhibition and IC₅₀ values of DPPH radical scavenging activity of synthesized CHME and its corresponding Sm(III) complex.

Compound	Concentration (µg/mL)				
	25	50	75	100	IC ₅₀
CHME	21.56	40.21	58.23	73.46	64.84
Sm(CHME) ₃ .bipy	21.38	42.27	58.12	80.47	61.77
Std.	34.02	56.22	76.12	92.01	43.78

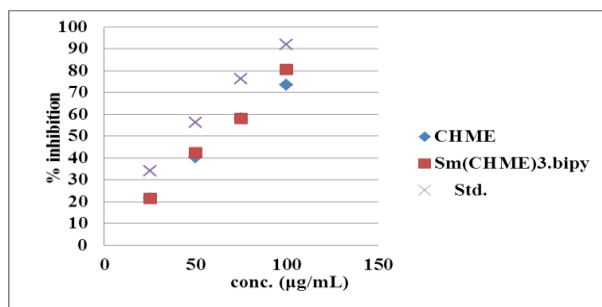


Fig. 2 Percentage inhibition values of CHME, Sm (CHME)₃. bipy with respect to standard ascorbic acid.

CONCLUSION

In conclusion, Sm (CHME)₃.bipy has been synthesized successfully by adopting solution precipitation method by employing 2-hydroxy-4-methoxy-2-(4-chlorophenyl) acetophenone (CHME) as main ligand and 2,2'-bipyridine as ancillary ligand. ¹H-NMR and infrared spectra indicated that the free ligand CHME was coordinated with Sm(III) ion through the phenolic hydroxyl group, carbonyl group and nitrogen atoms of the ancillary ligands. The ligand was found to be inactive against all the investigated microbes whereas tested complex exhibited better activity than standard ciprofloxacin against *S. aureus*.

ACKNOWLEDGEMENTS

Authors express their profound thanks to the University Grant Commission, New Delhi for providing financial

assistance in the form of a major research project, No.F.41-348/2012 (SR).

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