Antibacterial Co(II) Complexes of Benzothiazole-Derived Compounds

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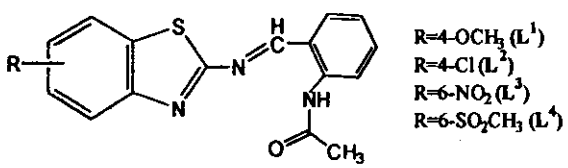
Summary: Reaction of 2-acetamidobenzaldehyde with 2-amino-4-methoxy-, 2-amino-4-chloro-, 2-amino-6-nitro- and 2-amino-6-methylsulfonyl benzothiazole afforded a series of congeners which have been further used for complexation reaction to obtain potential Co(II) compounds. The title compounds of the type $[M(L)_2]X_n$, where M=Co(II), L=L^1-L^4 of Fig.1, X=NO_3^-, SO_4^{2-}, $C_2O_4^{2-}$ or $CH_3CO_2^-$ and n=1 or 2, have been characterized by physical, spectral, and analytical data. The compounds act tridentately and their metal(II) complexes are proposed to possess an octahedral geometry about the central metal atom. These compounds in comparison to their metal complexes have been screened for antibacterial properties against the pathogenic species *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Introduction

Benzothiazoles and their derivatives are well known biologically active compounds [1-4]. Much research has been devoted to study their metallo-organic as well as biological properties [5-13]. Biological activity of the compounds is connected to their ability [14,15] to form complexes with the metal ions that lead to a "lock geometry" *via* coordination so that only certain substrates are able to become attached to this framework of interaction [16,17]. Anticancer and/or antibacterial drugs are known to be versatile ligands [18]. Recent studies have indicated that many compounds exhibit increased anticancer/ antibacterial activity when administered in the form as their metal complexes [19-21].

This interaction between the metal ion and such biologically active ligands represents an important route in designing new metal-based anticancer/ antibacterial therapies against different kinds of tumors and bacterial infections that become resistant to the use of conventional drugs [22,23]. These observations attracted the attention to synthesize and report [24,25] antibacterial benzothiazole-derived Schiff bases with Co(II), Ni(II) and Zn(II) metal ions. In order to understand in more detail, the biological role of anions [NO_3^-, SO_4^{2-}, $C_2O_4^{2-}$ or $CH_3CO_2^-$] on some of the same compounds (L^1-L^4) (Fig 1) *via* coordination with the cobalt(II) metal ion against pathogenic bacterial species *i.e.*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* is also reported. It is expected that this alteration may result in achieving new targets in synthesizing and designing metal-based more potential compounds that may fight more aggressively against those antibiotic/bactericidal, which become resistant to the common infectious bacterial strains.



(Fig. 1) Structure of the Ligands

Results and Discussion

Physical Properties

The compounds (L^1-L^4) were prepared by refluxing an equimolar amount (0.01 mmol) of 2-acetamidobenzaldehyde and substituted 2-aminobenzothiazoles in hot (40-50 °C) ethanol (30 mL). The structures of these compounds were established and reported elsewhere with the help of their IR, NMR, and microanalytical data [24,25]. These compounds were used for the complexation reaction with Co(II) metal ion. All of the newly synthesized metal(II) complexes [(1)-(16)] were air and moisture stable (Table I). They were prepared by the stoichiometric reaction of the corresponding metal(II) salts and the respective ligands in the molar ratio M : L of 1 : 2. The complexes are amorphous solids, which decompose above 200 °C. They are insoluble in common organic solvents such as

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Table I. Physical, Spectral and Analytical Data of the Metal(II) Chelates.

Metal Cehlate	M.P. (°C)	B.M. (μ_{eff})	Calc. (Found)% C	H	N	Yield (%) (%)
(1) $[Co(L^1)_2](NO_3)_2$ $C_{36}H_{30}CoN_8O_{10}S_2$	209-211	4.2	49.7 (49.9)	3.4 (3.5)	12.9 (12.5)	60
(2) $[Co(L^1)_2](SO_4)$ $C_{36}H_{30}CoN_6O_8S_3$	212-214	4.3	51.3 (51.0)	3.6 (3.8)	10.0 (10.4)	58
(3) $[Co(L^1)_2](C_2O_4)$ $C_{38}H_{30}CoN_6O_8S_2$	209-211	4.6	54.7 (55.1)	3.6 (3.5)	10.1 (10.5)	61
(4) $[Co(L^1)_2](CH_3CO_2)_2$ $C_{40}H_{36}CoN_6O_8S_2$	215-217	4.5	55.6 (55.8)	4.2 (4.7)	9.7 (9.5)	61
(5) $[Co(L^2)_2](NO_3)_2$ $C_{32}H_{24}CoClN_8O_8S_2$	206-208	4.7	53.4 (53.1)	3.3 (3.5)	15.6 (15.9)	60
(6) $[Co(L^2)_2](SO_4)$ $C_{32}H_{24}CoClN_6O_6S_3$	210-212	4.4	48.5 (48.9)	3.0 (3.4)	10.6 (10.5)	61
(7) $[Co(L^2)_2](C_2O_4)$ $C_{32}H_{24}CoClN_6O_6S_2$	212-214	4.6	49.0 (49.2)	3.1 (3.5)	10.7 (10.9)	62
(8) $[Co(L^2)_2](CH_3CO_2)_2$ $C_{36}H_{30}CoClN_6O_6S_2$	218-220	4.5	53.1 (53.4)	3.7 (3.2)	10.3 (10.1)	59
(9) $[Co(L^3)_2](NO_3)_2$ $C_{32}H_{24}CoN_{10}O_{12}S_2$	205-207	4.7	43.8 (43.6)	2.7 (3.0)	16.0 (16.4)	61
(10) $[Co(L^3)_2](SO_4)$ $C_{32}H_{24}CoN_8O_{10}S_3$	212-214	4.6	45.3 (45.5)	2.8 (2.4)	13.2 (13.4)	60
(11) $[Co(L^3)_2](C_2O_4)$ $C_{34}H_{24}CoN_8O_{10}S_2$	218-220	4.5	48.6 (48.3)	2.9 (2.5)	13.3 (13.7)	62
(12) $[Co(L^3)_2](CH_3CO_2)_2$ $C_{36}H_{30}CoN_8O_{10}S_2$	220-222	4.3	49.7 (49.4)	3.4 (3.0)	12.9 (12.6)	60
(13) $[Co(L^4)_2](NO_3)_2$ $C_{34}H_{30}CoN_8O_{12}S_4$	209-211	4.6	43.3 (43.9)	3.2 (3.5)	11.9 (11.5)	61
(14) $[Co(L^4)_2](SO_4)$ $C_{34}H_{30}CoN_6O_{10}S_5$	214-216	4.4	44.6 (44.3)	3.3 (3.0)	9.2 (9.0)	59
(15) $[Co(L^4)_2](C_2O_4)$ $C_{36}H_{30}CoN_6O_{10}S_4$	222-224	4.5	47.7 (47.9)	3.3 (3.5)	9.3 (9.6)	60
(16) $[Co(L^4)_2](CH_3CO_2)_2$ $C_{38}H_{36}CoN_6O_{10}S_4$	220-222	4.3	48.7 (49.0)	3.8 (3.4)	9.0 (9.3)	62

ethanol, methanol, chloroform or acetone, but soluble in DMSO and DMF. Molar conductance values of the soluble complexes in DMF showed value (82-95 $ohm^{-1}cm^2mol^{-1}$) indicating that they are electrolytes [26].

Infrared Spectra

IR spectra of the ligands showed the absence of bands at 1735 and 3420 cm^{-1} due to carbonyl ν(C=O) and $\nu(NH_2)$ stretching vibrations and, instead, a strong new band appeared at ~1635-1640 cm^{-1} assigned to the azomethine ν(HC=N) linkage [27]. This suggested that amino and aldehyde moieties of the starting reagents have been converted into their corresponding ligands (Fig. 1). The comparison of the IR spectra of these ligands and their Co(II) chelates indicated that the ligands are coordinated to the metal atom in three ways, thus representing the ligands acting in a tridentate manner (Table II).

The band appearing at 1635 cm^{-1} due to the azomethine group was shifted to lower frequency by ~10-15 cm^{-1} indicating participation of the azomethine nitrogen in the complexation [28].

The band at 1615 cm^{-1} assigned to the benzothiazole ring nitrogen, ν(C=N), also shifted to lower frequency by ~15-20 cm^{-1}, which is indicative of the involvement of the ring nitrogen of the benzothiazole moiety in chelation [27].

A medium-strong band appearing at 3190 cm^{-1} and assigned to ν(NH) remained unchanged, thus providing a clue that this NH group in not involved in the coordination. However, a band at 1685 cm^{-1} assigned to the amido group, CONH, in the ligand was shifted to lower frequency by 10-15 cm^{-1} indicating, in turn, the coordination of the amido oxygen to the metal atom [28].

Further conclusive evidence of the coordination of these ligands with the metal atom was shown by the appearance of new, weak low-frequency bands at 525 and 455 cm^{-1} assigned to the metal-nitrogen [ν(M-N)] and metal-oxygen [ν(M-O)]

Table-II:IR and Electronic Spectral Data of the Complexes

	IR (cm^{-1})	λ_{max} (cm^{-1})
(1)	3190(s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,535, 17,495, 30,170.
(2)	3190 (s, NH), 1625(s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,355, 17,280, 29,965.
(3)	3190 (s, NH), 1620 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,430, 17,505, 30,165.
(4)	3190 (s, NH), 1625 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,615, 17,425, 29,980.
(5)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,255, 17,255, 29,895.
(6)	3190 (s, NH), 1625 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,690, 17,645, 30,110.
(7)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,285, 17,250, 29,925.
(8)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,675, 17,510, 29,995.
(9)	3190 (s, NH), 1620 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,415, 17,255, 29,715.
(10)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,475, 17,515, 30,105.
(11)	3190 (s, NH), 1620 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,260, 17,470, 29,905.
(12)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,335, 17,495, 30,100.
(13)	3190 (s, NH), 1625(s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,455, 17,380, 29,910.
(14)	3190 (s, NH), 1620 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,630, 17,305, 29,815.
(15)	3190 (s, NH), 1620 (s, HC=N), 1605 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 ms, M-O).	8,445, 17,415, 30,265.
(16)	3190 (s, NH), 1625 (s, HC=N), 1600 (s, C=N), 1670, 1535 (s, CONH), 530 (ms, M-N), 455 (ms, M-O).	8,340, 17,385, 29,790.

s = sharp, ms = medium sharp.

stretching, respectively [28]. These new bands were observable only in the spectra of the metal complexes and not in the spectra of their uncomplexed ligands, thus confirming the participation of these hetero atoms (O or N) in the complexation.

NMR Spectra

The NMR spectra of the ligands and their diamagnetic Zn(II) complexes taken in DMSO-d_6 have already been reported [24,25]. However, the NMR spectra of the Co(II) complexes showed very broad resonances and, therefore, are not included. The reported ligands exhibited signals due to all the protons in their expected region and have been identified from the integration curve equivalent to the total number of protons deduced from the proposed structures. These were compared with the reported signals of the known comparable compounds and give further support for the composition of these new ligands as well as their Zn(II) complexes [29]. Comparison of the chemical shifts of the uncomplexed ligands with those of the corresponding Zn(II) complexes show that some of the resonance signals underwent a downfield shift of 0.9-1.0 ppm upon complexation indicating coordination of the ligands with the Zn(II) ion. ^{13}C NMR spectra likewise showed similar diagnostic features for the ligands as well as their Zn(II) complexes [29].

Magnetic Moments and Electronic Spectra

The geometry of the Co(II) complexes has been further deduced from their electronic spectral and magnetic moment data (Tables I and II). The room temperature magnetic moment of the solid cobalt(II) complexes was found in the range 4.2-4.7 B.M, indicative [30,31] of three unpaired electrons per Co(II) ion in an ideal octahedral environment.

The electronic spectra of the Co(II) complexes showed three bands observed at 8,255-8,690, 17,250-17,645 and 29,715-30,265 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^3A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transitions respectively, and are suggestive of an octahedral geometry around the cobalt ions [32,33].

On the basis of the above observations, it is suggested that the Co(II) complexes show an octahedral geometry in which the two ligands act as tridentates and accommodate themselves around the metal atom in such a way that a stable chelate ring of the complex is formed, hence giving a stable structure to the complex.

Antibacterial Properties

The title ligands and their Co(II) chelates were evaluated for their antibacterial activity against *Escherichia coli (a), Staphylococcus aureus (b), and Pseudomonas aeruginosa (c).* The compounds were tested at a concentration of 30 μg/0.01 mL in DMF solution using the paper disc diffusion method [34-38]. The diameter of the susceptibility zones was measured in mm and the results are reproduced in Table III. The susceptibility zones measured were the clear zones around the discs killing the bacteria.

All the ligands and their complexes individually exhibited varying degrees of inhibitory

Table-III: Antibacterial Activity Data of the Ligands and Complexes.

Ligand Complex	Microbial Species		
	a	b	c
L^1	+	++	++
L^2	++	++	+++
L^3	+++	+	++
L^4	++	++	++
(1)	+++	++++	++++
(2)	++++	+++	+++
(3)	+++	+++	+++
(4)	++	+++	+++
(5)	++++	++++	++++
(6)	+++	+++	+++
(7)	++	+++	+++
(8)	++	+++	++
(9)	++++	++++	+++
(10)	+++	++++	++
(11)	+++	+++	++
(12)	+++	++	++
(13)	++++	++++	+++
(14)	+++	++++	++++
(15)	++++	+++	++
(16)	+++	+++	+++
$Co(NO_3)_2.6H_2O$	+	+	+
$CoSO_4.6H_2O$		-	+
$CoC_2O_4.2H_2O$	+	+	-
$Co(CH_3CO_2)_2.4H_2O$	+	+	-

a = *Escherichia coli* b = *Staphylococcus aureus* c = *Pseudomonas aeruginosa.*

effects on the growth of the tested bacterial species. The antibacterial results evidently show that the activity of the uncomplexed ligands became more pronounced when coordinated to the metal ion. Generally, the cobalt(II) complexes showed enhanced activity (80-85 %) against all the three testing bacterial species. However, it was also evident that when the same metal chelate having different anions was individually screened, the degree of potency varied. From the obtained data reproduced in Table III, it was observed that the order of potency in comparison to the Co(II) complexes having chloride ion evaluated and reported earlier [24], and the results of the present studies against the same tested bacterial species under the same conditions were found to follow the order of potency as

$$NO_3^- > C_2O_4^{2-} > CH_3CO_2^- > Cl^- > SO_4^{2-}$$

On the basis of these observations, it is strongly claimed that different anions dominantly effect the biological behavior of the metal chelates [39-41]. It is, however, suspected that different factors such as solubility, conductivity and dipole moment influence the cell permeability mechanism, which in turn is effected by the presence of these anions present in the complex outside the coordination sphere [42,43]. These may be the possible reasons for increasing this potency.

The individual metal(II) salts, however, showed negligible activity (6-10 %) against the testing bacterial species.

Inhibition zone diameter mm (% inhibition): +, 6-10 (27-45 %); ++, 10-14 (45-64 %); +++, 14-18 (64-82 %); ++++, 18-22 (82-100 %). Percent inhibition values are relative to inhibition zone (22 mm) of the most active compound with 100 % inhibition.

Experimental

Material and Methods

All chemicals and solvents used were of A.R grade. Cobalt(II) salts were used as nitrate, sulfate, acetate and oxalate. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. UV-Visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrophotometer. C, H and N analyses were carried out by Butterworth Laboratories Ltd. Conductances of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurements were done on solid complexes using the Gouy method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Preparation of Ligand (L^1)

It was prepared by the reported method in which 2-amino-4-methoxybenzothiazole (1.4 g, 0.01 mmol) in ethanol (10 mL) was added to a hot ethanol solution (30 mL) of 2-acetamidobenzaldehyde (1.6 g, 0.01 mmol) [24,25]. Then 2-3 drops of conc. H_2SO_4 were added and the mixture refluxed for 2 h. On cooling, a solid product was formed, which was filtered, washed with ethanol, then with ether and dried. Crystallization from hot ethanol gave L^1. The same method was applied for the preparation of L^2-L^4 by using the corresponding reagents in the same molar ratio.

Preparation of Co(II) Complex

A warm ethanol solution (30 mL) of the ligand (0.02 mmol) was added to a magnetically stirred solution of the respective Co(II) salt (0.01 mmol) in ethanol (25 mL). The mixture was refluxed for 1 h and then cooled to room temperature. On

cooling to room temperature, a solid product precipitated. The product thus obtained was filtered, washed with ethanol, then with ether and dried. Crystallization from aqueous ethanol (30:70) gave the desired Co(II) complex.

Antibacterial Studies

Preparation of Discs

The ligand/complex (30 μg) in DMF (0.01 mL) was mounted on a paper disc [prepared from blotting paper (3 mm diameter)] with the help of a micropipette. The discs were left in an incubator for 48 h at 37 °C and then applied on the bacteria grown on agar plates.

Preparation of Agar Plates

Minimal agar was used for the growth of specific bacterial species. For the preparation of agar plates for *Escherichia coli,* MacConkey agar (50 g) obtained from Merck was suspended in freshly distilled water (1 L). It was allowed to soak for 15 minutes and then boiled on a water bath until the agar was completely dissolved. The mixture was autoclaved for 15 minutes at 120 °C and then poured into previously washed and sterilized Petri dishes and stored at 40 °C for inoculation.

Procedure of Inoculation

Inoculation was done with the help of a platinum wire loop, which was heated to red-hot in a flame, cooled and then used for the application of the bacterial strains.

Application of Discs

Sterilized forceps were used for the application of the paper discs on previously inoculated agar plates. When the discs were applied, they were incubated at 37 °C for 24 h. The zone of inhibition around the disc was then measured (in mm).

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