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SYNTHESIS AND CHARACTERIZATION OF BENZOTHIAZOLE SCHIFF BASES BASED METAL COMPLEXES AND THEIR BIOLOGICAL APPLICATIONS

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ABSTRACT

A new benzothiazole Schiff base ligand named *(E)-N*-(6-((2-hydroxy-5-methylbenzylidene)amino)benzo[d]thiazol-2-yl)cyclohexane-carboxamide (BSB) was prepared through the condensation reaction of *N*-(6-aminobenzo[d]thiazol-2-yl)cyclohexane-carboxamide with substituted aldehyde. Mass, FT-IR, NMR, and spectral techniques confirmed the synthesized ligand. The resulting compound was tested with the coordination behavior of a series of various d-block metal ions named Cu^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+}, with the prepared (BSB) reported. The bonding behavior and the complexes' performance have been assumed as part of the required characterization techniques. BSB complexes with various metals were extensively studied for cytotoxic activity against Vero, human breast cancer (MCF7).

Keywords: Ligand, Benzothiazole, Novel Schiff Bases, Metal Complexes, MCF7 Studies.
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INTRODUCTION

Imines are constructed organic buildings with a common molecular formula of $R_1R_2C=NR$.[1] Amination reactions of products were synthesized from the condensation of an aliphatic or aromatic amine with substituted carbonyl compounds. Schiff bases of aromatic substitutes are more stable than alkyl ones.[2] They are engaged in many divisions, including analytical, biological, and as a ligand with metal complexes in inorganic chemistry.[3] Most of the imine-based natural compounds have more promising biological activities than simple ligands.[4] Schiff base amino acid complexes have extraordinary importance in inorganic sustainability due to their biological activities.[5] Heterocycle derivatives based on benzothiazole act to help in the benzene ring fused with 4 and 5-positions for the respective thiazole rings, shown an extensive series of numerous chemical activities. Additionally, benzothiazole scaffolds with imine bases have several properties in the biological, analytical, medicinal, and inorganic sciences owing to their antimicrobial activities towards various pathogens.[6-8] Imine-like bases have been properly used in the development of inorganic metal complexes, meanwhile, they are also a common system for stable complexes showing a wide range of biological activities.[9] Additionally, benzothiazole derivatives comprising nitrogen and sulfur were shown various pharmacological activities[10] and anticonvulsant effects.[11] Coordination complexes of benzothiazole are identified to show antimicrobial activity against various bacteria.[12-15] phenylene groups 1,3 and 1,4s have been used to support the development of ligands adept at involving single- and double-stranded metal complexes.[16] Concentration studies of imine bases constrain the growth of *Staphylococcus aureus (S. aureus)* (MTCC 1144) bacteria.[17] Imine bases and their inorganic metal complexes were exposed tremendous activity against *S. aureus*, where complexes are additional active than their ligand complements.[18] Inorganic metal-incorporated supports have gained importance as a class of coordination compounds for their flexibility towards structural diversity, synthesis, and varied binding capabilities.[19-22] Imine-based renowned ligands that perform a critical role in coordinating metal and their binding efficiency and solubility.[23] Imine based metal complexes branches from their structural stability, chelating properties, and their wide biological activity.[24-26] Consider the structure-activity connection of imine base is vital for understanding their varied biological activities, making it a respected stage for designing and constructing effective drug molecules in the field of bioinorganic chemistry. [27-29] Based on the above result, a new benzothiazole-based Schiff base ligand named *N*-(6-{(*E*)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-benzothiazol-2-yl) cyclohexane carboxamide (BSB) was

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developed through a condensation reaction of normal amine and aldehydes. The synthesized ligand was used to form a metal complex with various transition metal ions, and MTT assay studies were conducted using this metal complex.

EXPERIMENTAL

Materials and Methods

N-(6-nitro-1,3-benzothiazol-2-yl) cyclohexane carboxamide (TCI chemicals 99%), hydrochloric acid, and Tin chloride ($SnCl_2$) (Spectrochem, 99%), 2-hydroxy-5-methylbenzaldehyde (TCI chemicals 99%), and Sodium hydroxide (Spectrochem, 99%) were purchased from TCI and Spectrochem chemicals. Furthermore, N, N-dimethylformamide (DMF, 99%), ethanol (EtOH, 99%), methanol (MeOH, 99%), acetonitrile (CH_3CN, 99%), and other solvents were purchased from commercial sources without further purification.

Apparatus and Analysis

Required chemicals are commercially purchased and used without further purification. Spectral data for ^1H and ^{13}C-NMRs were recorded in a 300 MHz Bruker spectrometer using DMSO-d_6 and $CDCl_3$ as solvents with TMS as the internal standard. Electron spray ionization mass spectrometry (ESI-MS) analysis was recorded in a Thermo Fisher instrument, in both negative and positive ion modes. The FT-IR technique was utilized in the model device (PerkinElmer Spectrum Version 10.02.00) to identify the functional groups of the different stages of the catalysts and reaction products in the range of 4000-800 cm^{-1}.

Synthesis of N-(6-amino-1,3-benzothiazol-2-yl)cyclohexanecarboxamide

A 100 ml round-bottom flask fitted with a magnetic stirrer was charged with the N-(6-nitro-1,3-benzothiazol-2-yl) cyclohexane carboxamide (3 g, 0.0098 mol) and 45 ml of Con. HCl. To the resulting reaction mass, $SnCl_2$ (5.57 g, 0.029 mol) was added at room temperature, and the reaction mass was stirred at RT for 6 hr. The progress of the reaction was monitored by TLC. After the reaction was completed, the reaction mass was slowly poured onto crushed ice and mixed with water. Then, the reaction mass was basified with 1M NaOH to a pH of 9-10. The off-white solid formed was extracted with ethyl acetate (2x 150). The organic layer was separated and washed with brine solution (1 × 200 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting solid product was purified by washing the residue with hexane to afford 2.12 g N-(6-amino-1,3-benzothiazol-2-yl)cyclohexane carboxamide. Pale yellow solid; Yield 72%; M.P. 251-252 °C; FT-IR (KBr) νmax in cm^{-1}: 3412 (-OH stretching), 3291 (NH Stretch, amine), 3135 (CH Stretch, Aromatic), 2917 (CH Stretch, Aliphatic), 1694 (C=O Stretch), 1604 (C=N Stretch), 1553 (NH bend), 1250 (C-N Stretch), 845 (CH, bending); ^1H -NMR (300 MHz, DMSO-d_6) δ ppm: 2.14 (s, 3H, methyl), 5.13 (s, 2H, amine), 6.70 (dd, 1H), 6.98 (d, 1H), 7.38 (d, 1H), 11.98 (s, 1H, amide); ^{13}C-NMR (300 MHz, DMSO-d_6) δ ppm: 22.61, 104.09, 114.38, 120.77, 132.86, 139.59, 145.62, 153.05, 168.62; LC-MS (ESI) m/z: 276.0472 $(M+H)^+$ in Fig.-S1 to S3 in supporting Information.

Synthesis of Schiff bases SB-1: N-(6-{(E)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-mbenzothiazol-2-yl)cyclohexanecarboxamide

A 100 ml round-bottom flask fitted with a magnetic stirrer and N_2 atmosphere was charged with the N-(6-amino-1,3-benzothiazol-2-yl)cyclohexane carboxamide 2 (1.5 g, 0.007 mol) and 40 mL of ethanol, then treated with 2-hydroxy-5-methylbenzaldehyde (1.08 g, 0.08 mol). The resulting reaction mass was heated under reflux for 14 h. The progress of the reaction was monitored by TLC. The reaction mixture was allowed to RT, and the resulting solid was filtered and recrystallized with methanol to afford 1.92 g of product as an orange solid; Yield 80%; Detail ^1H and ^{13}C-NMR spectra and LC-MS (ESI) m/z: 394.0735 $(M+H)^+$ for compound BSB in figure S4 to S6.in supporting information).

General Procedure for the Preparation of Metal Complexes

To a stirred solution of N-(6-{(E)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-benzothiazol-2-yl) cyclohexane carboxamide (0.3 g, 0.0007 mol) in ethanol (6 mL) was added a methanolic solution of metal salts: Cu $(NO_3)_2$.$6H_2O$, Zn$(NO_3)_2$.$6H_2O$, Co$(NO_3)_2$.$6H_2O$ and $NiCl_2$.$6H_2O$ (0.112 g, 0.113 g, 0.110 g, and 0.09 g respectively, 0.00038 mol) with warming condition and stirred for five hour metal complexes precipitates formed during cooling were filtered, washed using ethanol, and dried out in vacuum evaporator.

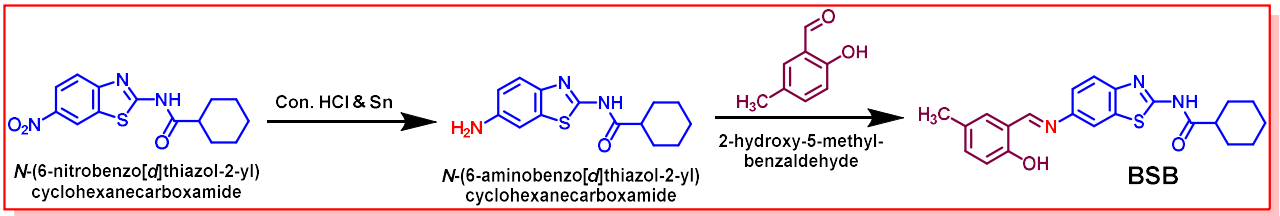
MTT Assay Studies

In the procedure, using a seed of 200µl cell suspension in a 96-well plate required a respective cell density (20,000 cells per well) without using any test agent. The cells are permitted to develop for 24 hours. Then add appropriate concentrations of the test agents, followed by plate incubation for about 24 h at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were taken out from the incubator, and the media was supplemented with MTT reagent to a final concentration of 0.5mg/mL of total volume. The plates to the incubator and incubated for 3 hours. Remove the MTT reagent using 100µl of solubilization solution like DMSO. The gyratory shaker was enhanced dissolution, pipetting up and down may be required to completely dissolve the MTT formazan crystals, especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength. Finally, % Cell viability is calculated using the following formula:

Percentage cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100

RESULTS AND DISCUSSION

A new benzothiazole-based Schiff base ligand named *N*-(6-{(*E*)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-benzothiazol-2-yl) cyclohexane carboxamide (BSB) was prepared through condensation reaction of *N*-(6-amino-1,3-benzothiazol-2-yl)cyclohexane carboxamide react with substituted aldehydes in 1:1 molar ratio detail scheme was discussed in Scheme-1.



N-(6-nitrobenzo[*d*]thiazol-2-yl) cyclohexanecarboxamide

N-(6-aminobenzo[*d*]thiazol-2-yl) cyclohexanecarboxamide

Scheme-1: synthesis of *N*-(6-{(*E*)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3 benzothiazol-2-yl)cyclohexanecarboxamide (BSB)

Spectral Studies

Synthesized BSB structure was confirmed by FT-IR spectra (Fig.-1), which shows that the peak at 3425 cm^{-1} indicates the presence of –OH group, the peak at 3281 cm^{-1} confirms the presence of –NH group in BSB, the ketone peak was observed at 1686 cm^{-1} due to conjugation from amine to ketone, and also 1602 cm^{-1} confirmed to the formation of –C=N peaks respectively.

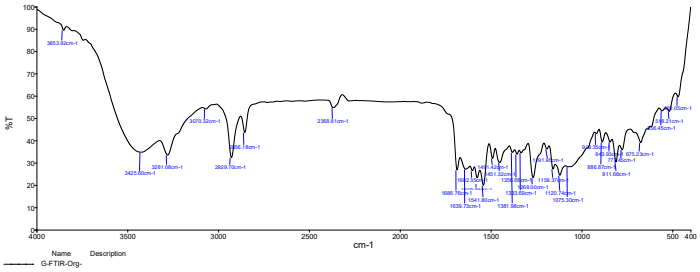


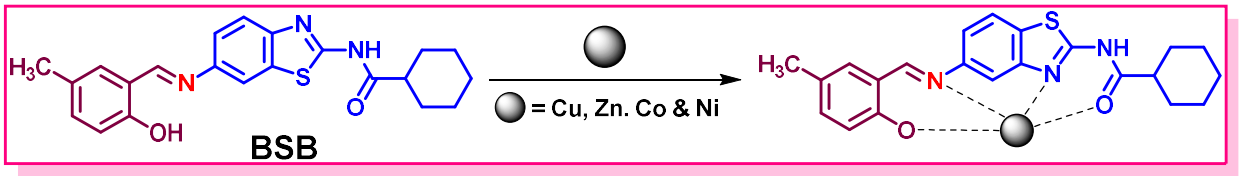
Fig.-1: FT-IR Spectra for BSB

The ^{1}H-NMR (400 MHz, DMSO-d_6) spectra of the BSB ligand (Fig.-S1 in supporting information) showed two singlet peaks. The first was observed at δ = 1.0 to 2.5 ppm, respectively, which was assigned to the aliphatic protons. The second peak at δ_{ppm} = 12.3 and 12.8, respectively, was assigned to the Schiff base proton (-HC=N-). The peak at 9.0 ppm corresponds to an amine proton. The sets of peaks observed in the range of 6 to 8.5 ppm were ascribed to the aromatic rings. Two broad singlet peaks at δ = 12.01 ppm were attributed to the hydroxyl proton of BSB. The ^{13}C-NMR resonances of the BSB (Fig.-S2 in supporting information) carbonyl carbon occurred at δ = 175.16 ppm, imine of benzothiazole ring (-C=N-), and the Schiff base carbon atom (-HC=N-) were observed at δ = 158.42 and 158.04 ppm, respectively. The peaks at δ = 162.53 were assigned to the one carbon linked to the hydroxyl groups (-C-OH). The unsubstituted

aromatic carbons were observed in the range of 114 to 147 ppm. The aliphatic carbons were observed in the range of 19.94 to 43.51 ppm, respectively. Further, the ligand was confirmed from LC-MS analysis (Fig.-S3 in supporting information), which represents the 394.0735 [M+H] adduct for BSB.

Synthesis of Metal Complexes

A metal complex was prepared by using N-(6-{(E)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-benzothiazol-2-yl) cyclohexanecarboxamide (0.3 g, 0.0007 mol) in ethanol (6 mL) was added a methanolic solution of metal salts: Cu $(NO_3)_2$.$6H_2O$, $Zn(NO_3)_2$.$6H_2O$, $Co(NO_3)_2$.$6H_2O$ and $NiCl_2$.$6H_2O$ (0.112 g, 0.113 g, 0.110 g, and 0.09 g respectively, 0.00038mol) with warming condition and stirred for 5 hr. Metal complexes precipitate formed during cooling were filtered, washed using ethanol, and dried in a vacuum evaporator in Scheme-2.



Scheme-2: Synthesis of Metal Complexes and their General Structure

FT-IR Studies for Metal Complexes

Metal complexes of BSB with various transition metal ions were confirmed from FT-IR spectra, which were obtained in the presence of Fig.-2.

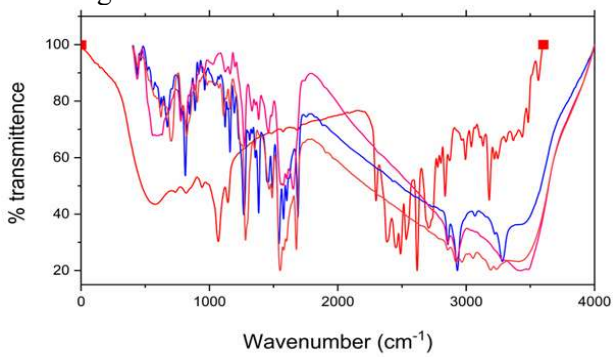


Fig.-2: FT-IR Spectra for Ligand BSB with Various Transition Metal Ions (Cu, Zn, Co & Ni)

The FTIR spectral bands of the BSB and its complexes are shown in Table-2. The FTIR spectrum of free BSB shows sharp bands at 1686 and 1602 cm^{-1}, which were assigned to the stretching vibration of ketone (–C=O) and imine (-CH=N-), respectively. However, these bands may overlap with ν(C=C), which makes their complete interpretation difficult [18]. The ketone and imine peaks were shifted to higher frequency upon complexation with metal ions. Moreover, the low-frequency regions of the spectra indicate the appearance of two weak-intensity bands in the range of 431 to 598 cm^{-1} in the FTIR spectra of the complexes, which were assigned to metal-nitrogen vibrations ν(M-N).

Table-1: FT-IR Main Stretching Absorption Bands (cm^{-1}) of BSB and its Metal Complexes

Code	-C=O stretching	-C=N-, Benzothiazole	-C-OH stretching	-C-NH stretching	M-N-	M-N-
BSB	1686	1602	3425	3281		
BSB@Cu	1703	1617	3426	3266	517	438
BSB@Zn	1688	1601	3281	3223	465	437
BSB@Co	1652	1601	3424		592	437
BSB@Ni	1677	1600	3404	3236	622	438

LC-MS Studies for BSB with Various Metal Complexes

In LC-MS analysis for BSB, the complex of BSB with various transition metals was validated from LC-MS analysis for Cu, Zn, Co, and Ni metals. Initially, in mass observed at 394.03 [M+H] peak was observed

after the reaction with copper, a peak at 394.03 disappeared, and the new peak appeared at 849.37 $[M+H]^{+,}$ which corresponds to the formation of Cu@BSB (Fig.-S7 in supporting information). The same procedure was followed for Zn, Co, and Ni metals, and the detailed confirmation mass spectra were included in Fig.-7 in the supporting information.

The Cytotoxic Studies against MCF-7

The transition metal complexes with BCB were treated against MCF-7 cells to determine the cytotoxicity by using an MTT assay that measures mitochondrial dehydrogenase activity as an indication of cell viability. After 24 h of incubation, the compounds can arrest the proliferation of the corresponding tumor cells. The results were studied by the cell viability curves and correlated with the IC_{50} values of the studied concentration range from 0.1-100 µM. The cytotoxic assay against MCF7 cell lines under various metal complexes using BSB with Co, Cu, Zn, and Ni transition metals is displayed in Fig.-3.

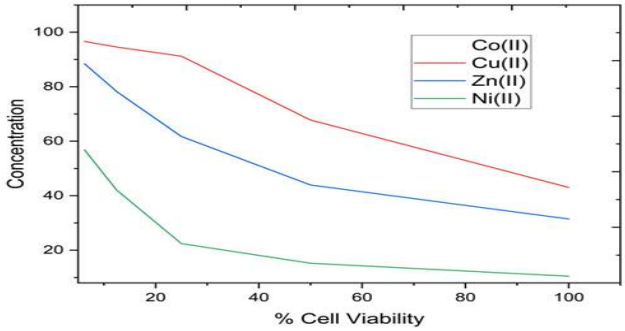


Fig.-3: Cell Viability Studies of Ligand BSB with Metal Complexes like Co, Cu, Zn, and Ni

IC_{50} values can be calculated for a given compound by determining the concentration needed to prevent half of the maximum biological response. The cytotoxicity of such Co, Cu, Zn, and Ni metal complexes detail studies was detailed in Fig.-S8 in the supporting information. The IC_{50} values for various metal complexes with BSB were included in Table-2.

Table-2: IC50 value for BSB@ Various Metal Complexes

Metal Complexes	IC_{50} value (µg/mL)
Co(II)	89.78
Cu(II)	93.71
Zn(II)	57.37
Ni(II)	10.21

Table-2 indicates that the Ni complex acted at a minimal concentration for cell viability compared to other metal complexes. IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonists on reversing agonist activity. Anticancer cell line testing with MCF 7 studies indicated that the Ni(II) complex arrested the cell cycle efficiently and promoted tumor cell destruction via a reactive oxygen species (ROS)-mediated mitochondrial pathway. The Ni(II) complexes show great activity towards the MCF-7 cell line.

CONCLUSION

The present work, a new benzothiazole-based Schiff base ligand named *N*-(6-{(*E*)-[(2-hydroxy-5-methylphenyl)methylidene]amino}-1,3-benzothiazol-2-yl) cyclohexane carboxamide (BSB) was prepared through condensation reaction of *N*-(6-amino-1,3-benzothiazol-2-yl)cyclohexane carboxamide react with substituted aldehydes in a 1:1 molar ratio. FT-IR, Mass, NMR, and spectral techniques characterized the synthesized ligand. The resulting compound was tested with the coordination behavior of a series of transition metal ions, named Cu(II), Zn(II), Co(II), and Ni(II), with the prepared Schiff base ligand (BSB) reported. The nature of bonding and the behavior of complexes have been deduced from FT-IR, UV-Vis, NMR, and spectral studies. The synthesized BSB-based metal complexes were elaborately performed for cytotoxicity activity against Vero, human breast cancer (MCF7).

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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