East African Journal of Biophysical and Computational Sciences

Journal homepage : https://journals.hu.edu.et/hu-journals/index.php/eajbcs

Synthesis, Characterization and Antibacterial activity of Benzimidazole Derivatives and their Cu (ii), Ni (ii) and Co (ii) complexes

Haftom Welderufael*, Dagne Addisu Kure, Endalkachew Asefa Moges, Lelisa File, Salah Hamza Sherif

Department of Chemistry, Hawassa University, Hawassa, Ethiopia

KEYWORDS:

Antibacterial;
Benzimidazole;
Metal complex;
Schiff base

Research article

ABSTRACT

Benzimidazole is one of the privileged nitrogen-containing heterocyclic compounds, which is found in many bioactive compounds, benzimidazole and its derivatives have evolved as an important heterocyclic system due to their potency in a wide range of biologically active compounds like anthelmintic, antibacterial, antifungals, anti-inflammatory, antiviral, and so on. Derivatives of 1-arylsulfonylbenzimidazole and their respective Cu (II), Ni (II) and Co (II) complexes were successfully synthesized. The structures of all the synthesized ligands were confirmed by using IR, UV-Visible, ^{1}H NMR, and ^{13}C NMR spectroscopy. The Cu (II), Ni (II) and Co (II) complexes were confirmed by using IR and VU-Visible spectra. The IR spectra of ligands and its metal complexes imply that the benzimidazol derivative ligands behave as basic bidentate ligands coordination through the azomethine nitrogen and oxygen atom. *In-vitro* antibacterial activity of all the synthesized ligands and their metal complexes were evaluated by using disc diffusion method against *K. pneumoniae, E. coli,* and *S. aureus* bacterial species .The tested compounds and metal complexes exhibited from good to excellent activity (zone of inhibition (ZI) ranged 10 mm to 23 mm). Compound BIL1 exhibited better activity than the standard drug against *E. coli* (ZI of 15 mm) and *K. pneumoniae* (ZI of 5 mm) compared with gentamycin ((ZI of 15mm). Complex CoC exhibited better activity against *S. aureus* (ZI of 23 mm) compared with *gentamicine* (ZI value of 21 mm). This compound is a good starting point to develop new drug for treating pathogenic diseases. Therefore, synthesis of more analogue were recommended for further discovery of a new drug candidate.

INTRODUCTION

Microbial resistance is one of the critical public health issues and the greatest challenges of the twenty-first century (Marinescu, 2021) especially as increasing numbers of strains are becoming resistant to multiple antimicrobial agents, with some bacteria now being resistant to all available antibiotics, there is an pressing need to develop new drugs with novel mechanisms of action (Fatmah *et al.*, 2015). Nitrogen-heterocycles play a vital role in medicinal chemistry and they have been intensively used as scaffolds for drug development, among nitrogen containing heterocyclic compounds, benzimidazole is continuously drawing the interest of many researchers for the

*Corresponding author:
Email: haftish1@gmail.com, 251 926046602

https://dx.doi.org/10.4314/eajbcs.v4i1.4S

development of newer drug moiety (Majumder *et al*., 2013).

Benzimidazole derivatives are known to possess varied biological activities, substituted benzimidazole derivatives have been reported to possess antimicrobial (Ansari *et al*., 2009; Özkay *et al*., 2010; Shaharyar *et al*., 2017), anti-inflammatory (Mariappan *et al*., 2015; Achar *et al*., 2017), and antiviral (Zou *et al*., 1996; S. Hirashima *et al*., 2006; Bhagdev, K. and Sarkar, 2021), anticancer (Błaszczak-S´ *et al*., 2014; Sharma *et al*., 2017; Ting-Ting *et al*, 2018) activities. The structural similarity of the benzimidazole moiety to naturally occurring nucleotides makes it a valuable drug scaffold in medicinal chemistry. The strong link between the benzimidazole core and a wide range of biological activities is well-documented and established in the literature. Benzimidazole derivatives have also been used as good ligands for transition metal ions due to the large conjugated pi-system and the azomethine nitrogen which can positively affect the structures of the complexes (Galal *et al*., 2010).

Due to their potent biological activity, metal complexes with aromatic Schiff base ligands, particularly those based on imidazoles, have gained considerable interest in recent years (Kumaravel *et al*., 2017). The biological activities of several metal complexes with benzimidazole ligands have been created and investigated (Horacio *et al*., 2008; Kopel *et al*., 2015; Ashraf *et al*., 2016; Kumaravel and Raman 2017).

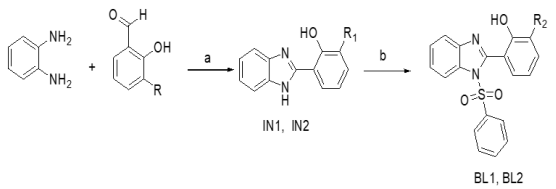
In this paper, the synthesis and antibacterial activity investigations of benzimidazole derivatives and their metal complex against *K. pneumonia, E. coli*, and *S. aureus* bacterial strains were presented. These bacteria were selected on the basis that E. coli known to cause Urinary tract infections, *K. pneumoniae* know to be the causative agent of pneumonia and *S. aureus* is multi drug resistant (Abioye *et al*., 2013). Therefore, the objective of this study was to compare the activity of the ligands with their metal complexes.

MATERIALS AND METHODS

All reagents and solvents were of analytical grade and used as received. Reaction mixtures were monitored via thin-layer chromatography (TLC) on silica gel plates. Column chromatography employing silica gel (100–200 mesh) was primarily used for purification of the intended products. Melting points were determined using open capillary tubes and are reported uncorrected. Infrared (IR) spectra were collected on an FT-IR Bruker Alpha spectrometer. Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker Advance NMR spectrometer operating at 400 MHz, with tetramethylsilane (TMS) as the internal standard.

Chemistry

The benzimidazole derivatives was prepared as outlined in scheme 1, the intermediate, 2-substituted benzimidazole was prepared by refluxing o-phenylendiamine with appropriately substituted benzaldehyde in Dimethyl formamide (DMF) in the occurrence of $NaHSO_4$ as a catalyst, the benzene sulphonyl substituted compounds were prepared by stirring 2-substituted benzimidazole derivatives with benzene sulphonyl chloride at room temperature in acetone in the presence of sodium carbonate.



IN1, R_1 = OCH_3, IN_2, R_1= H; BIL1, R_2 = OCH_3, BIL2, R_2 = H

SCHEME 1: reagent and condition: a) $NaHSO_3$, DMF, reflux 90-100^oC; b) K_2CO_3 acetone, RT

Synthesis

Synthesis of 2-(1H-benzo[d]imidazol-2-yl)-6-methoxyphenol(IN1)

A mixture of o-phenylendiamine (1.09g, 10mmol) and (1.248g, 10mmol) sodium hydrogen sulphite were dissolved in 15 mL dimethyl formamide (DMF) stirred at room temperature for 30min, to this solution (1.52g, 10mmol) 2-o-vanillin were added, the mixture was refluxed for about 6hr at 90^{o}C-100^{o}C, the development of the reaction was regulated by TLC. At the end of the reaction, the reaction mixture was cooled and extracted with ethyl acetate. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting material was purified by column chromatography using a 9:1 mixture of hexane and ethyl acetate.

Synthesis of 2-(1H-benzo[d]imidazol-2-yl) phenol (IN2)

A mixture of *o*-phenylendiamine (0.54g, 5mmol) and (0.624g, 5mmol) of sodium hydrogen sulphite were dissolved in 15 mL dimethyl formamide (DMF) stirred at room temperature for 30min, to this mixture (0.605g, 5mmol) salisaldehyde were added and the mixture was refluxed for about 6hrs. at 90^{o}C-100^{o}C,the progress of the reaction was regulated by TLC. At the end of the reaction, the reaction mixture was cooled and extracted with ethyl acetate. The organic phase was harvested and dried over anhydrous Na_2SO_4; the solvent used was removed under reduced pressure recrystallized from methanol.

Synthesis of N-benzenesulfonyl-2-(1H-benzo[d]imidazol-2-yl)-6-methoxyphenol (BIL1)

2-(1H-benzo[d]imidazol-2-yl)-6-methoxy phenol (IN1) (0.38g, 1.77mmol) were dissolved in 10 mL acetone and (0.614g, 4.429mmol) K_2CO_3 were added and stirred at room temperature for 30min, then 0.3 mL of benzene sulfonyl chloride was added drop wise to the mixture and refluxed for 6 hours, The reaction's progress was followed by thin-layer chromatography (TLC). Once complete, the reaction mixture was partitioned between water and ethyl acetate. The organic phase was collected, dried over anhydrous sodium sulfate (Na_2SO_4), and concentrated in vacuo. The resulting product was then subjected to column chromatography, using a 9:1 mixture of hexane and ethyl acetate as the mobile phase.

Synthesis of N-benzenesulfonyl-2-(1H-benzo[d]imidazol-2-yl) phenol (BIL2)

2-(1H-benzo[d]imidazol-2-yl)phenol(IN2) (1.15g, 5.4 mmol) was dissolved in 10 mL of acetone,K_2CO_3(1.8g, 13.5mmol) were added

and stirred at room temperature for 30min, 0.8 mL of benzene sulfonyl chloride were added to the mixture drop wise and refluxed for 6 hours, The progress of the reaction was monitored by TLC. At the end of the reaction, the reaction mixture was cooled and extracted with ethyl acetate. The organic phase was collected and dried on anhydrous Na_2SO_4 and crystallized under abridged pressure. The product was then further purified by column chromatography.

Synthesis and Characterization of metal complexes of BIL1

A methanolic solution of ligand BIL1 (0.38 g, 0.5 mmol, in 10 mL) was combined with a separate methanolic solution (5 mL) of either $Cu(CH_3COO)_2 \cdot H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, or $CuCl_2 \cdot 6H_2O$ (1 mmol each). The combined solutions were refluxed for 8 hours, with the reaction being monitored by thin layer chromatography (TLC). After allowing the mixture to cool in an ice bath, the precipitate was collected by filtration and washed with dichloromethane. The solid was dried under vacuum overnight, and then recrystallized from methanol.

Antibacterial activities

Culture media and disk preparation

Nutrient agar, Muller Hinton agar and Nutrient broth were prepared according to the manufacturer instruction in which the prepared media was autoclaved at 121^{o}C for 15 minutes. Then the prepared culture media was checked for the sterility for 24 hours at 37 ^{o}C. For quality control, strains of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*), which were obtained from the College of medicine and health science of Hawassa University, known American type culture collection committee (ATCC) were used to perform the antibacterial activities of the agents. Whatman filter paper 41 is used to prepare a disk of 5mm diameter using manual paper punching.

Preparation of chemical solution and media for the antibacterial activity

By using analytical balance, a 0.005g of each chemical powder was added to 15µl dimethyl sulphoxide (DMSO) and mixed to form a homogenous solution. A 5µl of the solution was added to the sterile disk prepared before using sterile micropipette. The aforementioned bacterial strains (meant for quality control) were inoculated on sterile nutrient agar plates using sterile loop. The streaked plates were incubated for 24 hours at 37^{o}C. A 3 to 5 colonies were picked with sterile loop and suspended in 5 mL nutrient broth to form standards. The culture suspension was then inoculated on sterile Muller Hinton agar plate using sterile cotton swab in three directions to get uniform inoculum. The antibiograms profiles of the test organisms to the control antibiotic were checked. Then an autoclaved disks with a control gentamicin and solution impregnated disks were carefully positioned on the plate and incubated for 24 hours at 37^{o}C. The unique ID number of each disk was mentioned with permanent marker on the back of the Petri dishes. After incubation the diameter of the zone of inhibition was measured using ruler. The result was given in (table 1)

RESULT AND DISCUSSION

Characterization

Characterization of 2-(1H-benzo[d]imidazol-2-yl)-6-methoxyphenol (IN1)

Light red solid; Yield:76.1%;Mp:195-197 ^{o}C; IR(KBr, Cm^{-1}): 3394(-NH), 3240(-OH), 3047(C-H, aromatic), 2854(C-H, methyl), 1593(C=N); ^{1}H-NMR (DMSO-d_6,400MHz): δ_H 13.12(s, 1H,-OH), 7.91-789(m, 2H, Ar-H), 7.73-763(m, 2H, Ar-H), 7.42-7.37(m, 2H, Ar-H), 7.26-7.25(m, 1H, Ar-H), 5.00(s,1H,-NH), 3.7(s,3H,OCH_3); ^{13}C-NMR (DMSO-d_6, 100MHz): δ_C 149.2, 147.1, 142.6, 137.0, 123.5, 122.8, 121.1, 120.1, 114.3, 111.0, 42.7

Electronic spectra of compound IN1

The UV-Vis spectrum of the IN1 compound was recorded in DMSO. The electronic absorption spectrum of the ligand showed bands at 304 nm, attributed to a π-π* transition of the –C=C– bond; 382 nm, corresponding to an n-π* transition; and 497 nm, assigned to an n-π* transition of the azomethine chromophore (–C=N–).

Characterization of N-benzenesulfonyl-2-(1H-benzo[d]imidazol-2-yl)-6-methoxypheno1 (BIL1)

Light red solid; Yield:68.0%, Mp:230-232^{o}C; IR(KBr, Cm^{-1}): 3210 (-OH), 2923(C-H, aromatic), 2854(C-H), 1458(C=N), 1377(O=S=O); ^{1}HNMR(DMSO-d_6,400MHZ): δ_H 13.2 (s,1H,OH), 7.9(d, *J*= 8.4Hz,2H), 7.7(m, 2H), 7.6(d, *J*=7.4Hz,2H), 7.5(m,1H), 7.4(d, *J*=6.5Hz,1H), 7.3(m,2H), 7.1(d, *J*=7.65Hz,1H), 6.9(m,1H,), 3.7(s,3H, OCH_3);^{13}C-NMR (DMSO-d_6, 100MHz): δ_C 153.1, 147.6, 136.0, 133.8, 129.8, 128.3, 126.9, 126.4, 123.0, 122.7, 122.5, 115.6, 115.0, 56.4

Electronic spectra of compound BIL1

The UV/Visible spectral for BIL1 compound recorded in DMSO, the electronic absorption spectrum of the ligands showed band at 302nm attributed to –C=C-,$\pi \rightarrow \pi^*$ transition, the band around 382nm and 495nm is because of n$\rightarrow \pi^*$ transition of the(-C=N-) azomethine chromophore.

Characterization of N-benzenesulfonyl-2-(1H-benzo[d]imidazol-2-yl) phenol (BIL2)

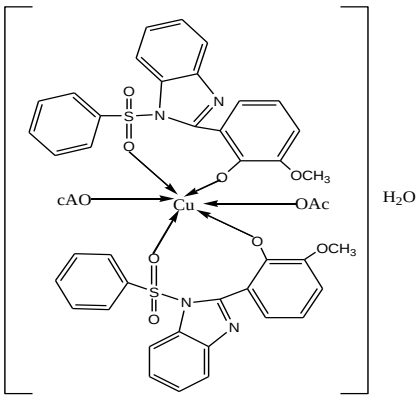
Yellow solid; Yield:71.2 %, Mp:196-198 ^{o}C, IR(KBr, Cm^{-1}): 3232 (O-H), 2954(C-H, aromatic), 1458(C=N),1176 (C-O) strong band at1377 (O=S=O); ^{1}H-NMR(400MHz, DMSO-d_6)δ_H:13.15(s, 1H, -OH), 7.74-7.61(m, 5H, Ar-H), 7.29-7.20(m, 4H, Ar-H), 7.08-7.06 (m, 2H, Ar-H), 6.97-6.91(m, 2H, Ar-H); ^{13}C-NMR (100 MHz,DMSO-d_6) δ_C:115.3, 118.1, 119.5, 121.3, 121.52, 121.94, 122.64, 123.0, 123.33, 128.9, 131.1, 137.9, 138.9, 141.5, 155.3

Characterization of Cu(II) complexes of BIL1(CuC)

The product was obtained as a light brown solid with a 74.5% yield and a melting point of 214-216 °C. The IR spectrum (KBr, cm^{-1}) showed characteristic peaks at 3394 (O-H stretching of water), 2923-2858 (C-H), 1604 (C=N), and 1377 (S=O). The C-O stretch, observed at a higher frequency in the Cu (II)

complexes compared to the free ligands, suggests logical coordination through the deprotonated phenolic-O. This is supported by the disappearance of the weak phenolic OH band in the complex spectra. Far-infrared bands corresponding to M-O bonds, including a band at 1188 cm^{-1} assigned to the (Cu-O) bond in the complexes, further corroborate the proposed coordination mode of the Schiff base ligands.

The Uv/visible spectra recorded in DMSO, the electronic absorption spectrum of the ligands showed band at 301nmattributed to $\pi \rightarrow \pi^*$ transition of –C=C-, the band around 382nm and 502nmis due to n→ π^* transition of the (-C=N-) azomethine chromophore.



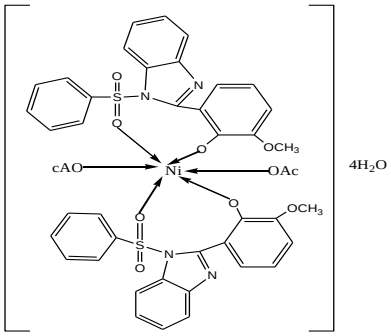
Proposed structure of Cu(II) complex of BIL1(CuC)

Characterization of Ni(II) complexes of BIL1(NiC)

Pale green solid, yield 76.9%, Mp: 220-222^oC; IR (KBr, Cm^{-1}):3240 (O-H stretching of water), 2923-2858(C-H, stretching), 1604(C=N), 1461 (C-O), 1377 (S=O). The C-O stretch of the free ligands was observed at a higher frequency in the spectra of the Ni(II) complexes, suggesting coordination of the Schiff base ligands through the deprotonated phenolic–O, This was substantiated by the disappearance of the weak phenolic OH band in the spectra of the complexes. The mode of coordination of the Schiff base ligands was further corroborated by the appearance of bands in the far-infrared spectra of the complexes due to the M-O bonds. The new bands in the low frequency region 510 and 440 are due to the formation of (M–O) and (M–N) vibrations respectively.

Electronic spectra of compound NiC

The Uv/visible spectral for compound NiC are recorded in DMSO the data are presented in the electronic absorption spectrum revealed band at 302nm attributed to $\pi \rightarrow \pi^*$transition of (–C=C) ,the band around 382nm and 499nm is due to$\pi \rightarrow \pi^*$and n→ π^* transition of the (-C=N-) azomethine chromophore.



Proposed structure of Ni(II) complexof BIL1(NiC)

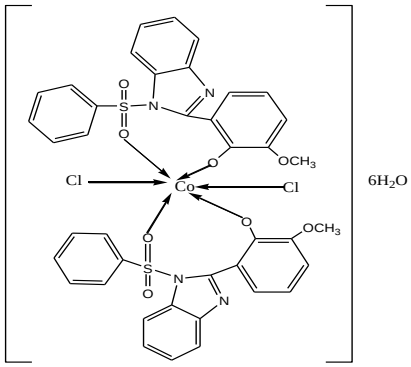
Characterization of Co (II) complexes of BIL1 (CoC)

Yellow solid, Yield 88.8%, Mp:210-212^oC, The IR stretching (KBr, *v* in cm^{-1}) spectral information for compound CoC, showed distinguishing medium band at 3394cm^- confirmed the presence of O-H stretching of water. Strong band at 2923-2858cm^{-1}

guaranteed the presence of C-H stretching of aromatic. A medium band at 1604cm^{-1} confirmed the availability of C=N stretching of azomethine. A medium narrow band at 1461cm^{-1}confirmed the presence of C-O stretching of aromatic. A medium narrow band at 1677cm^{-1} confirmed the presence of C=C stretching aromatic and the strong band at 1377cm^{-1} confirmed the presence of two S=O stretching of benzene sulfonyl. A higher C-O stretching frequency was observed in the infrared spectra of the Co(II) complexes compared to the free ligands, which indicates coordination of the Schiff base ligands via the deprotonated phenolic oxygen. The conclusion made based on these findings was supported by the absence of the weak phenolic O-H band in the complex spectra. Moreover, the far-infrared spectra of the complexes showed bands that can be assigned to M-O vibrations, including one at 1130 cm^{-1} corresponding to the Co-O bond.

Electronic spectra of compound CoC

The Uv/visible spectra for compound CoC were noted in DMSO, the electronic absorption spectrum of the ligands showed band at 267nm associated with –C=C- ,$\pi \rightarrow \pi^*$ transition, the band around 302nm and 319nm is because of $\pi \rightarrow \pi^*$and n$\rightarrow \pi^*$ transition of the-C=N-azomethine chromophore. No spectral bands were found below 300nm which supports octahedral geometry.



Proposed structure of Co (II) complex of BIL1(CoC)

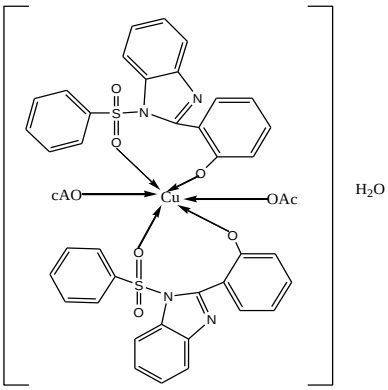
Characterization of copper (II) complexes of BIL2 (CuC2)

The light brown solid, CuC2, was obtained with an 86.7% yield and a melting point of 160-162 °C. The IR spectrum (KBr, cm^{-1}) exhibited a medium band at 3363 cm^{-1} indicative of O-H stretching from water, strong bands at 2923-2858 cm^{-1} due to aromatic C-H stretching, a medium band at 1612 cm^{-1} consistent with C=N stretching in the azomethine, a medium narrow band at 1461 cm^{-1} corresponding to aromatic C-O stretching, a medium narrow band at 1917 cm^{-1} assigned to aromatic C=C stretching, and a strong band at 1377 cm^{-1} suggesting two S=O stretching in the benzene sulfonyl group. A shift to higher frequency in the C-O stretching band was observed for the Co(II) complexes compared to the free ligands, indicating coordination of the Schiff base ligands through deprotonated phenolic oxygen. This observation was substantiated by the absence of the characteristic phenolic OH band in the complex spectra. Additionally, the far-infrared spectra of the complexes exhibited

bands that can be attributed to M-O vibrations, including one at 1170 cm^{-1} corresponding to the Co-O bond.

Electronic spectra of compoundCuC2

The Uv/visible spectral for compound CuC2 are recorded in DMSO. The data are offered in the electronic absorption spectrum of the ligands showed band at 274nm (36496cm^{-1}) attributed to –C=C-,$\pi \rightarrow \pi^*$transition the band around 300nm and 332nm due to $\pi \rightarrow \pi^*$and n→ π^*transition of the (-C=N-) azomethine chromophore. No spectral bands were found below 10000cm^{-1} which supports octahedral geometry.



Proposed structure of Cu(II) complex of BIL2 (CuC2).

Table-1: In-vitro antibacterial activity values of the synthesized benzimidazol derivatives and their Cu(II), Ni(II) and Co(II) complexes

		Zone of inhibition (in mm)		
Entry	**Compounds**	***E. coli***	***S. aureus***	***K. pneumonia***
1	IN1	5	10	5
2	BIL 1	15	18	8
3	BIL2	5	7	5
4	CuC $[Cu(BIL1)_2(OAc)_2].H_2O$	11	18	8
5	NiC $[Ni(BIL1)_2(OAc)_2].4H_2O$	10	20	6
6	CoC $[Co(BIL1)_2(Cl)_2].6H_2O$	12	23	11
7	CuC2 $[Cu(BIL2)_2(OAc)_2].H_2O$	5	5	5
Standard	Gentamycin	14	21	5

The synthesized benzimidazol derivative and Cu(II), Ni(II) and Co(II) complexes were tested for *in-vitro* antibacterial activity against *E. coli, S. aureus* and *K. pneumonia*. Gentamicin was used as standard antibacterial drug. The zone of inhibition values clearly showed that all the compounds exhibited a varied range 5-23mm (table 1) against all the tested bacterial strains. Compounds IN, BIL2 and CuC2 had less activity against *E. coli* and *S. aureus*. Compounds NiC and CuC showed moderate antibacterial activity against *E. coli* and *S. aureus.* Compound BIL1 exhibited better activity against *E. coli* (15mm zone of

inhibition) compared to standard (14mm zone of inhibition) but moderate activity against *S. aureus*; compound CoC exhibited better activity against *S. aureus* (23mm zone of inhibition) compared to standard (21mm zone of inhibition) but moderate activity against *E. coli.*

CONCLUSION

All the synthesized compounds in this study showed better activity against *K. pneumonia* compared to standard. Comparing the two benzimidazole derivatives BIL1 and BIL2; BIL1 showed better activity against all the tested bacterial strain, structurally the two compounds are differ by the presence of methoxy substituent at 3 position of phenyl ring, it can be concluded that the dramatic increase in the activity is due to the presence of this substituent. This compound is a good starting point to develop new drug for treating pathogenic diseases. Therefore, synthesis of more analogue were recommended for further discovery of a new drug candidate.

Acknowledgement

The authors thank Addis Ababa University for running the NMR, IR, and UV-vis spectra and Hawassa University for providing research funding.

References

Ashraf A., Siddiqui W. A., Akbar J., Mustafa G., Krautscheid H., Ullah N., Mirza B., Sher F., Hanif M. and Hartinger C.G. 2016. Metal complexes of benzimidazole derived sulfonamide: Synthesis, molecular structures and antimicrobial activity. *Inorganica Chimica Acta* **443**: 179–185.

Arpi M., Ragini G. and Anshu J. 2013. Microwave-assisted, synthesis of nitrogen-containing heterocycles. *Green Chemistry Letters and Reviews* **6**:2, 151-182,

Bhagdev K. and Sarkar S. 2021. Benzothiazole Moiety and Its Derivatives as Antiviral Agents. *Med. Sci. Forum*. 7 (1): 1-9

Fatmah A.S. Alasmary, Snelling A.M., Zain M.E., Alafeefy A.M., Awaad A.S. and Karodia N. 2015. Synthesis and Evaluation of Selected Benzimidazole Derivatives as Potential Antimicrobial Agents. *Molecules* **20**(**8**): 15206–15223,

Kumaravel G. and Raman N. 2017. A treatise on benzimidazole based Schiff base metal(II) complexes accentuating their biological efficacy: Spectroscopic evaluation of DNA interactions, DNA cleavage and antimicrobial screening Ganesan Kumaravel. *Mater. Sci. Eng*. **70**: 184–194

Mariappan G., Hazarika R., Alam F., Karki R., Patangia U. and Nath S. 2015. Synthesis and biological evaluation of 2-substituted benzimidazole derivatives. *Arab. J. Chem*. **8**: 715–719

Błaszczak-Swiatkiewicz K., Olszewska P. and Mikiciuk-Olasik E. 2014. Biological approach of anticancer activity of new benzimidazole derivatives. *Pharmacol Rep*. **66**: 100–106

Kavitha A., Kallappa H. and Harisha S. 2010. In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives, *Eur. J. Med. Chem*. **45**: 2048–2054

Ansari K.F. and Lal C.. 2009. Synthesis, physicochemical properties and antimicrobial activity of some new benzimidazole derivatives. *Eur. J. Med. Chem*. **44**: 4028–4033

Horacio L.S., Londonõ-Lemos M. E., Raúl G.-V., Israel P.M. , Pilar G.M., Isabel G.M. and Norah N.B. 2008. Synthesis, structure and biological activities of cobalt (II) and zinc (II) coordination compounds with 2-benzimidazole derivatives. *J. Inorg. Biochem*. **102**: 1267–1276

Ting-Ting M., Xu-Bing T., Dong-Dong L., Chen H., Xiao-Yan J., Geng Y., Shi-Fa W. and Gu W. 2018. Synthesis and biological evaluation of 2-aryl-benzimidazole derivatives of dehydroabietic acid as novel tubulin polymerization inhibitors. *RSC Adv*. **8**: 17511–17526

Marinescu, M. 2021. Synthesis of Antimicrobial Benzimidazole–Pyrazole Compounds and Their Biological Activities. *Antibiotics*. **10**: 1002, 1-29

Sharma P., Reddy T.S., Kumar N.P., Senwar K.R., Bhargava S.K. and Shankaraiah N. 2017. Conventional and microwave-assisted synthesis of new 1Hbenzimidazole-thiazolidinedione derivatives: A potential anticancer scaffold. *Eur. J. Med. Chem*. **138**: 234-245

Kopel P., Wawrzak D., Langer V., Cihalova K., Chudobova D., Vesely R., Adam V. and Kizek R. 2015. Biological Activity and Molecular Structures of Bis(benzimidazole) and Trithiocyanurate Complexes. *Molecules.* **20**: 10360-10376

Zou R., Ayres K.R., Drach J.C. and Townsend L.B. 1996. Synthesis and Antiviral Evaluation of Certain Disubstituted Benzimidazole Ribonucleosides. *J. Med. Chem.* **39**: 3477–3482

Galal S.A., Hegab K.H., Hashem A.M. and Youssef N.S. 2010. Synthesis and antitumor activity of novel benzimidazole-5-carboxylic acid derivatives and their transition metal complexes as topoisomerease II inhibitors. *Eur. J. Med. Chem*. **45**: 5685-5691

Hirashima S., Suzuki T., Ishida T., Noji S., Yata S., Ando I., Komatsu M., Ikeda S. and Hashimoto H. 2006. Benzimidazole Derivatives Bearing Substituted Biphenyls as Hepatitis C Virus NS5B RNA-Dependent RNA polymerase Inhibitors: Structure−Activity Relationship Studies and Identification of a Potent and Highly Selective Inhibitor JTK-109. *J. Med. Chem.* **49**: 4721–4736

Shaharyar M., Mazumder A. and Abdullah M. 2017. Synthesis, characterization and antimicrobial activity of 1,3,4-oxadiazole bearing 1H-benzimidazole derivatives. *Arab. J. Chem.* **10**: 503–508

Özkay Y., Tunal Y., Karaca H. and Isíkdag I. 2010. Antimicrobial activity and a SAR study of some novel benzimidazole derivatives bearing hydrazone moiety. *Eur. J. Med. Chem*. **45**: 3293-3298.

.