Synthesis, cytotoxic and antimicrobial activities of novel cobalt and zinc complexes of benzimidazole derivatives

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ABSTRACT

In this study fourteen novel cobalt (II) or zinc (II) complexes of benzimidazoles were synthesized from the 1-(4-substitutedbenzyl)-1H-benzimidazoles and $CoCl_2 \cdot 6H_2O$ or $ZnCl_2$. Cytotoxic activities of novel complexes were investigated against lung cancer cells (A549) and BEAS-2B. Three of the examined compounds (**1**, **4** and **5**) showed high cytotoxic activity against A549. While the IC_{50} of the cisplatin was 2.56 µg/mL for A549 cells at 72 h, the IC_{50} values of compounds **1**, **4** and **5** were 1.97, 1.87 and 1.9 µg/mL, respectively. IC_{50} values of these compounds for BEAS-2B cells were higher than the IC_{50} values for A549. While the IC_{50} values for BEAS-2B cells were 59.8, 24.5 and 32.67 µg/mL, respectively, the IC_{50} of the cisplatin was determined as 2.53 µg/mL in the present work. Three of the compounds have also high antimicrobial activity against all the microorganisms used.

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1. Introduction

Benzimidazole derivatives are interesting heterocycles because they are present in many naturally occurring products and various drugs. They also an important pharmacophore in modern drug discovery and continue to be the most versatile class of compounds possessing different pharmacological activities such as antitumor [1–3], antiulcer [4,5], antifungal [6,7], antibacterial [8–10], antihelmintic [11], anti-inflammatory [12–14], anticonvulsant [15], antitubercular [16–18], antidepressant [19], antihypertensive [20,21], anticoagulant [22] and antiviral [23,24].

Moreover, benzimidazole is of a considerable interest as a ligand toward transition metal ions with a variety of biological molecules including ionheme systems, vitamin B_{12} and its derivatives, and several metalloproteins. For this reason, the complexes of transition metal salts with benzimidazole derivatives have been extensively studied as a model structure of some important biological molecules. Metal complexes of biological important ligands are sometimes more active than free ligands [25]. It has been reported that the transition metal complexes with a ligand containing benzimidazole bearing trimethylsilylpropyl exhibit high antitumor activity [26]. Among the cancer types, lung cancer is a common cause of cancer-related death worldwide [27]. It is responsible from 1.3 million deaths annually across the world. In Turkey, the age-standardized incidence of lung cancer was found to be 75.8/100,000 population in men and 9.6/100,000 population in women [28]. Its incidence and mortality is 52.5/100,000 and 48.7/100,000 per year in the world, respectively [29]. Lung cancer is heterogeneous and consists of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [30]. The most common subtype NSCLC is responsible from nearly 80 to 90% of all lung cancers [29,31–33]. Despite novel molecular therapies, the 5-year relative survival rate of patients diagnosed with NSCLC is only 17% [29,32,34]. Conventional treatments of NSCLC are fairly ineffective [31]. Chemotherapy resistance is one of the main reasons leading to the failure of chemotherapy [35]. Therefore, there is an urgent need for novel drugs with an improved efficacy against NSCLC.

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In our previous works, some benzimidazole metal (cobalt(II), cooper (II), iron (II), nickel (II) and zinc (II)) complexes have been synthesized successfully and their physical and spectroscopic properties including single crystal x-ray analysis results reported [36—38]. More recently, antitumor properties of some benzimidazolium ligands were determined and promising results obtained [39].

According to the literature knowledge and our previous studies [36—40], in this study it was aimed to synthesized new cobalt(II) and zinc(II) complexes incorporating benzyl-substituted benzimidazole complexes (Scheme 1) in attempt to obtain possible active compounds having cytotoxic activities against cancer cell lines A549 and BEAS-2B and antimicrobial activities against Gram positive (*S. aureus* and *E. faceium*) and Gram negative bacteria (*E. coli* and *P. aeruginosa*) and a yeast, *Candida albicans*.

2. Experimental

The starting materials and reagents used in the reactions were supplied commercially by Acros, Aldrich or Merck Chemical Co. ^{1}H NMR (300.13 MHz) and ^{13}C NMR (75.47 MHz) spectra were recorded using a Bruker Avance 300 MHz Ultrashield high performance digital FT NMR spectrometer. Infrared spectra were recorded using an ATR unit from on a Perkin-Elmer FT-IR spectrophotometer. UV-Vis spectra were measured on a Perkin-Elmer Lambda 35 spectrophotometer. Elemental analyses were performed with a LECO CHNS-932 elemental analyzer. Melting points were recorded using an electrothermal-9200 melting point apparatus and are uncorrected.

1-(4-Chlorobenzyl)-1*H*-benzimidazole (**I**), 1-(4-bromobenzyl)-1*H*-benzimidazole (**II**), 1-(4-tolylbenzyl)-1*H*-benzimidazole (**III**), 1-(4-cyanobenzyl)-1*H*-benzimidazole (**IV**), 1-(4-methoxybenzyl)-1*H*-benzimidazole (**V**), 1-(4-nitrobenzyl)-1*H*-benzimidazole (**VI**), 1-(4-vinylbenzyl)-1*H*-benzimidazole (**VII**) were synthesized according to the literature procedures [41—46]. Compounds (**1—14**) were synthesized from 1-(4-substitutedbenzyl)-1*H*-benzimidazole with ZnCl$_2$ and CoCl$_2$ × 6H$_2$O in EtOH (Scheme 1).

2.1. Synthesis

2.1.1. Synthesis of dichlorobis[1-(4-chlorobenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (1)

A mixture of 1-(4-chlorobenzyl)-1*H*-benzimidazole (2.00 g, 8.24 mmol), ZnCl$_2$ (0.56 g, 4.12 mmol) and EtOH (20 mL) was heated under reflux for 4 h and then the mixture was cooled to room temperature and filtered off. White colored solid was crystallized with DMF/EtOH (1:2) (10 mL). White crystals of the title compound were washed with diethyl ether/*n*-hexane (1:1) and dried under ambient atmosphere. Yield: 2.25 g, 88%, m.p.: 257—258 °C. Anal. Calcd for C$_{28}$H$_{22}$Cl$_4$N$_4$Zn (%): C, 54.09; H, 3.57; N, 9.01. Found (%): C, 53.96; H, 3.63; N, 8.89. IR: $\nu_{(C=N)}$: 1489 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5,65 (s, 4H, CH$_2$), 7.28—7.43 (m, 12H, Ar-H), 7.61—7.67 (m, 2H, Ar-H), 7.84—7.90 (m, 2H, Ar-H), 8.90 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.9 (CH$_2$), 112.2, 118.9, 123.8, 124.3, 129.3, 130.0, 133.2, 133.3, 135.5, 140.8 (Ar-C), 145.5 (NCHN) ppm.

Similarly, the compounds of **3**, **5**, **7**, **9**, **11** and **13** were obtained by the reaction of 1-(4-bromobenzyl)-1*H*-benzimidazole, 1-(4-tolylbenzyl)-1*H*-benzimidazole, 1-(4-cyanobenzyl)-1*H*-benzimidazole, 1-(4-methoxybenzyl)-1*H*-benzimidazole, 1-(4-nitrobenzyl)-1*H*-benzimidazole, 1-(4-vinylbenzyl)-1*H*-benzimidazole with ZnCl$_2$ and **2**, **4**, **6**, **8**, **10**, **12** and **14** with CoCl$_2$ x 6H$_2$O, respectively.

2.1.2. Dichlorobis[1-(4-clorobenzyl)-1H-benzimidazole-$_K$N^3]cobalt (II), (2)

Yield: 2.28 g (blue crystals), 90%, m.p.: 254—255 °C. Anal. Calcd for C$_{28}$H$_{22}$Cl$_4$N$_4$Co (%): C, 54.66; H, 3.60; N, 9.11. Found (%): C, 53.90; H, 3.57; N, 8.83. IR: $\nu_{(C=N)}$: 1489 cm^{-1}.

2.1.3. Dichlorobis[1-(4-bromobenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (3)

Yield: 2.26 g (white crystals), 91%, m.p.: 263—264 °C. Anal. Calcd for C$_{28}$H$_{22}$Br$_2$Cl$_2$N$_4$Zn (%): C, 47.32; H, 3.12; N, 7.88. Found (%): C, 47.20; H, 3.08; N, 7.92. IR: $\nu_{(C=N)}$: 1491 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5,61 (s, 4H, CH$_2$), 7.27—7.35 (m, 8H, Ar-H), 7.53—7.64 (m, 6H, Ar-H), 7.82—7.86 (m, 2H, Ar-H), 8.80 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.9 (CH$_2$), 112.0, 119.1, 121.7, 123.5, 124.1, 130.3, 132.2, 133.4, 136.1, 141.3 (Ar-C), 145.3 (NCHN) ppm.

2.1.4. Dichlorobis[1-(4-bromobenzyl)-1H-benzimidazole-$_K$N^3] cobalt (II), (4)

Yield: 2.14 g (blue crystals), 87%, m.p.: 262—263 °C. Anal. Calcd for C$_{28}$H$_{22}$Br$_2$Cl$_2$N$_4$Co (%): C, 47.76; H, 3.15; N, 7.96. Found (%): C, 47.07; H, 3.20; N, 7.73. IR: $\nu_{(C=N)}$: 1491 cm^{-1}.

2.1.5. Dichlorobis[1-(4-tolylbenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (5)

Yield: 2.13 g (white crystals), 82%, m.p.: 238—239 °C. Anal. Calcd for C$_{30}$H$_{28}$Cl$_2$N$_4$Zn (%): C, 62.03; H, 4.86; N, 9.64. Found (%): C, 61.27; H, 4.91; N, 9.62. IR: $\nu_{(C=N)}$: 1483 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.25 (s, 6H, CH$_3$) 5,57 (s, 4H, CH$_2$), 7.13—7.32 (m, 12H,

I R = Cl
II R = Br
III R = CH$_3$
IV R = CN
V R = OCH$_3$
VI R = NO$_2$
VII R = CH=CH$_2$

1 R = Cl, M = Zn   **8** R = CN, M = Co
2 R = Cl, M = Co   **9** R = OCH$_3$, M = Zn
3 R = Br, M = Zn   **10** R = OCH$_3$, M = Co
4 R = Br, M = Co   **11** R = NO$_2$, M = Zn
5 R = CH$_3$, M = Zn   **12** R = NO$_2$, M = Co
6 R = CH3, M = Co   **13** R = CH=CH$_2$, M = Zn
7 R = CN, M = Zn   **14** R = CH=CH$_2$, M = Co
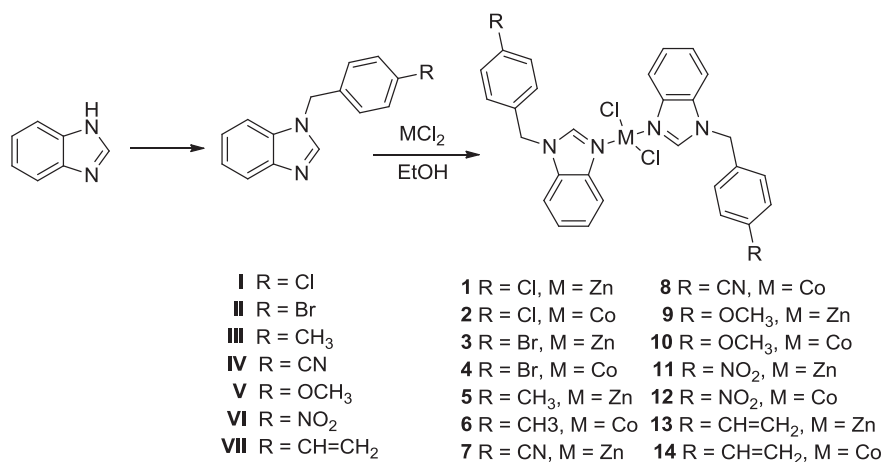
Scheme 1. Synthesis pathways of the benzimidazole complexes.

Ar-H), 7.61–7.64 (m, 2H, Ar-H), 7.82–7.85 (m, 2H, Ar-H), 8.81 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6,75.5 MHz): δ 21.1 (CH$_3$), 48.5 (CH$_2$), 112.2, 118.9, 123.6, 124.1, 128.1, 129.8, 133.4, 133.5, 137.8, 140.9 (Ar-C), 145.3 (NCHN) ppm.

2.1.6. Dichlorobis[1-(4-tolylbenzyl)-1H-benzimidazole-$_K$N^3]cobalt (II), (6)

Yield: 2.20 g (blue crystals), 85%, m.p.: 235–236 °C. Anal. Calcd for C$_{30}$H$_{28}$Cl$_2$N$_4$Co (%): C, 62.73; H, 4.91; N, 9.75. Found (%): C, 61.74; H, 4.84; N, 9.59. IR: $v_{(C=N)}$: 1483 cm^{-1}.

2.1.7. Dichlorobis[1-(4-cyanobenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (7)

Yield: 2.35 g (white crystals), 91%, m.p.: 213–214 °C. Anal. Calcd for C$_{30}$H$_{22}$Cl$_2$N$_6$Zn (%): C, 59.77; H, 3.68; N, 13.94. Found (%): C, 59.62; H, 3.63; N, 13.81. IR: $v_{(C=N)}$: 1482, $v_{(C≡N)}$: 2233 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.73 (s, 4H, CH$_2$), 7.29–7.85 (m, 16H, Ar-H), 8.78 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.6 (CH$_2$), 110.7 (CN), 111.4, 118.5, 118.7, 123.2, 123.7, 128.3, 132.7, 132.9, 140.7, 141.7 (Ar-C), 145.0 (NCHN) ppm.

2.1.8. Dichlorobis[1-(4-cyanobenzyl)-1H-benzimidazole-$_K$N^3]cobalt (II), (8)

Yield: 2.40 g (blue crystals), 94%, m.p.: 198–200 °C. Anal. Calcd for C$_{30}$H$_{22}$Cl$_2$N$_6$Co (%): C, 60.42; H, 3.72; N, 14.09. Found (%): C, 60.68; H, 3.98; N, 13.53. IR: $v_{(C=N)}$: 1482, $v_{(C≡N)}$: 2233 cm^{-1}.

2.1.9. Dichlorobis[1-(4-methoxybenzyl)-1H-benzimidazole-$_K$N^3] zinc (II), (9)

Yield: 2.30 g (white crystals), 90%, m.p.: 210–212 °C. Anal. Calcd for C$_{30}$H$_{28}$Cl$_2$N$_4$O$_2$Zn (%): C, 58.79; H, 4.60; N, 9.14. Found (%): C, 57.47; H, 4.28; N, 8.75. IR: $v_{(C=N)}$: 1485 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.70 (s, 6H, OCH$_3$) 5.54 (s, 4H, CH$_2$), 6.90 (d, 4H, J = 6.6 Hz, Ar-H), 7.28–7.36 (m, 8H, Ar-H), 7.65–7.68 (m, 2H, Ar-H), 7.82–7.85 (m, 2H, Ar-H), 8.80 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.7 (OCH$_3$), 55.0 (CH$_2$), 111.7, 114.1, 118.4, 123.0, 123.5, 127.9, 129.2, 132.8, 140.5, 158.9 (Ar-C), 144.1 (NCHN) ppm.

2.1.10. Dichlorobis[1-(4-methoxybenzyl)-1H-benzimidazole-$_K$N^3] cobalt (II), (10)

Yield: 2.23 g (blue crystals), 87%, m.p.: 208–209 °C. Anal. Calcd for C$_{30}$H$_{28}$Cl$_2$N$_4$O$_2$Co (%): C, 59.42; H, 4.65; N, 9.24. Found (%): C, 58.90; H, 4.55; N, 9.17. IR: $v_{(C=N)}$: 1485 cm^{-1}.

2.1.11. Dichlorobis[1-(4-nitrobenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (11)

Yield: 2.16 g (yellow crystals), 85%, m.p.: 303–304 °C. Anal. Calcd for C$_{28}$H$_{22}$Cl$_2$N$_6$O$_4$Zn (%): C, 52.32; H, 3.45; N, 13.07. Found (%): C, 52.04; H, 3.31; N, 12.89. IR: $v_{(C=N)}$: 1483 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.81 (s, 4H, CH$_2$), 7.29–7.35 (m, 4H, Ar-H), 7.53–7.62 (m, 6H, Ar-H), 7.87–7.90 (m, 2H, Ar-H), 8.17–8.22 (m, 4H, Ar-H), 8.90 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.4 (CH$_2$), 111.5, 118.6, 123.3, 123.8, 123.9, 128.6, 132.8, 140.5, 143.6, 147.1 (Ar-C), 145.1 (NCHN) ppm.

2.1.12. Dichlorobis[1-(4-nitrobenzyl)-1H-benzimidazole-$_K$N^3]cobalt (II), (12)

Yield:2.05 g (green crystals), 82%, m.p.: 300–301 °C. Anal. Calcd for C$_{28}$H$_{22}$Cl$_2$N$_6$O$_4$Co (%): C, 52.85; H, 3.48; N, 13.21. Found (%): C, 52.45; H, 3.26; N, 13.14. IR: $v_{(C=N)}$: 1482 cm^{-1}.

2.1.13. Dichlorobis[1-(4-vinylbenzyl)-1H-benzimidazole-$_K$N^3]zinc (II), (13)

Yield: 2.06 g (white crystals), 80%, m.p.: >350 °C. Anal. Calcd for C$_{32}$H$_{28}$Cl$_2$N$_4$Zn (%): C, 63.54; H, 4.67; N, 9.26. Found (%): C, 63.14; H,

4.58; N, 9.05. IR: $v_{(C=N)}$: 1484 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.25 (dd, 2H, $^3J_{cis}$ = 10.8, $^2J_{gem}$ = 0.9 Hz, CH=CH$_2$), 5.61 (s, 4H, CH$_2$), 5.80 (dd, 2H, $^3J_{trans}$ = 17.7, $^2J_{gem}$ = 0.9 Hz, CH=CH$_2$), 6.69 (dd, 2H, $^3J_{cis}$ = 10.8, $^3J_{trans}$ = 17.7 Hz, CH=CH$_2$), 7.28–7.30 (m, 4H, Ar-H), 7.33 (d, 4H, J = 8.4 Hz, Ar-H), 7.44 (d, 4H, J = 8.1 Hz, Ar-H), 7.61–7.64 (m, 2H, Ar-H), 7.82–7.85 (m, 2H, Ar-H), 8.82 (s, 2H, NCHN). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 47.9 (CH$_2$), 111.7 (CH=CH$_2$), 135.9 (CH=CH$_2$), 114.8, 118.4, 123.2, 123.7, 126.5, 127.9, 132.9, 135.6, 136.8, 140.4 (Ar-C), 144.9 (NCHN) ppm.

2.1.14. Dichlorobis[1-(4-vinylbenzyl)-1H-benzimidazole-$_K$N^3]cobalt (II), (14)

Yield: 1.98 g (blue crystals), 77%, m.p.: >350 °C. Anal. Calcd for C$_{32}$H$_{28}$Cl$_2$N$_4$Co (%): C, 64.23; H, 4.72; N, 9.36. Found (%): C, 64.05; H, 4.79; N, 9.29. IR: $v_{(C=N)}$: 1483 cm^{-1}.

2.2. Cell lines and culture conditions

The human cancer lines, lung adenocarcinoma (A549) and healthy human lung bronchial epithelium cells (BEAS-2B) were used for in vitro screening experiments. A549 cells were obtained from Prof. Dr. Fikrettin Sahin (Yeditepe University, Department of Genetics and Bioengineering, Istanbul/Turkey). The cells were maintained in RPMI 1640 growth medium containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in 5% CO$_2$ [47].

2.3. Drug treatment and MTT assay for cytotoxicity of compounds

Stock solutions of the benzimidazole metal complexes were prepared in dimethylsulfoxide (DMSO) and further dilutions were made with a fresh culture medium (the concentration of DMSO in the final culture medium was <0.1%). Cytotoxic effects of compounds against the cells were determined by MTT cell proliferation assay. Briefly, the A549 and BEAS-2B cells were plated in 96 well plates (5 × 10^3 cells/well) for 24 h before treatment with compounds to allow the attachment of the cells to surface of the plate. They incubated at 37 °C in a humidified incubator with 5% CO$_2$ for 24, 48 and 72 h. Then, the tested compounds were added to obtain the final concentration in the range of (0–100 μg/mL) and the cells were incubated for 24, 48 and 72 h. After incubation, the cells were treated with 20 μL of MTT reagent for 4 h at 37 °C. Then, the supernatants were removed from the plates and the MTT crystals obtained were homogenized by adding 100 μL DMSO into each well. To homogenize the pellets more efficiently, the plates were shaken for 5 min by shaker. Afterwards, the plates were read under 570 nm wavelengths by Microplate reader (Versa Max Tunable) [48]. Twelve wells were used for every concentration was repeated in twelve wells and IC$_{50}$ values (μg/mL) were defined as the compound concentrations reducing absorbance to 50% of control values. Cisplatin was also used as a control agent.

2.4. Microorganisms used

The microorganisms used for testing the antibacterial and antifungal activity were Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, Enterococcus faecium NJ-1 and Candida albicans, respectively. These microorganisms are stock cultures in the Biotechnology Laboratory, Inonu University. Stock cultures of these bacteria and yeast were maintained on blood agar and sabouraud dextrose agar at +4 °C, respectively.

2.5. Antimicrobial activity testing

The minimum inhibitory concentration (MIC) method was performed to test the antibacterial and antifungal activity of the newly synthesized compounds. Because dimethylsulfoxide (DMSO) has no inhibitory effect on growth of microorganisms in the concentration used, the stock solutions of the compounds were prepared by dissolving them in DMSO. The MICs of these compounds were investigated in sterile 96-well microplate by serial dilution with distilled water. The plates were incubated at 37 °C for 24 h under static conditions. The lowest concentration of the compounds at which no visible growth was seen was considered as the MIC.

2.6. Statistical analysis

Experiments were carried out for twelve wells. The results were expressed as mean ± SD. Statistical analysis was performed using GraphPad Prism 5. The difference between two groups was analyzed by 2 way Anova and one way Anova and Nonparametric Tukey test. A difference with or $P < 0.001$ (*) or $P < 0.05$ (**) was considered statistically significant.

3. Results and discussion

3.1. Synthesis and characterization of the benzimidazole compounds

The cobalt (II) and zinc (II) complexes were synthesized from the reaction of the appropriate benzimidazole ligand and metal salt. The complexes were smoothly crystallized in a DMF/EtOH mixture. The structures of Zn(II) benzimidazole complexes (**1**, **3**, **5**, **7**, **9**, **11** and **13**) were elucidated by ^{1}H NMR, ^{13}C NMR and IR spectrometric analyses. All spectral data were in agreement with the proposed structures. Due to paramagnetic properties of Co(II)-benzimidazole complexes (**2**, **4**, **6**, **8**, **10**, **12** and **14**) we could not observed appropriate NMR signals even more scans in diluted solvents. For this reason, the structures of Co(II) benzimidazole complexes were elucidated by IR and UV-Vis spectrometric and elemental analyses. IR spectra of the compounds show that the strong $\nu_{(C=N)}$ bands in free benzimidazoles at 1489-1495 cm^{-1} shift to 1482-1491 cm^{-1} for Co(II) and Zn(II) complexes. The bathochromic shift indicates the coordination of tertiary nitrogen of the ligands to Co (II) and Zn(II) atoms. These type of bathochromic shifts are also reported in the literature [25,36–38,49,50]. IR spectra of compound **IV** showed the $\nu_{(C\equiv N)}$ band at 2227 cm^{-1}, and this band shifted to 2233 cm^{-1} for corresponding Co(II) and Zn(II) complexes. IR spectra of compound **VI**, showed absorption band at 851, 1338 and 1509 cm^{-1} assigned to nitro group attached to 4-position of the benzyl group. The bands were observed at 858, 1344 and 1515 cm^{-1} for the corresponding Co (II) complex (**12**) and 858, 1344 and 1518 cm^{-1} for the corresponding Zn(II) complex (**11**). The nitro group frequencies shifted slightly higher frequency after coordinating the metal atom.

The characteristic CH resonances for the proton at position 2 of the benzimidazole ligand of Zn(II) complexes (**1**, **3**, **5**, **7**, **9**, **11** and **13**) were observed between 8.78 and 8.90 ppm.

As expected, the coordination to the Zn(II) ion shifts the ^{1}H NMR signals of the complex downfield from those of the free ligands ($\Delta\delta \approx$ 0.77- to 0.96 ppm) for the proton at position 2 of the benzimidazole ring. The protons of methylene in Zn(II) complexes were not shifted significantly to downfield ($\Delta\delta \approx$ 0.10−0.39 ppm).

The carbon resonances for the carbon at position 2 of the benzimidazole ligands of Zn(II) complexes (**1**, **3**, **5**, **7**, **9**, **11** and **13**) were observed between 141.1 and 145.5 ppm. The coordination to the Zn(II) ion shifts the ^{13}C NMR signals of the complex downfield

from those of the free ligands ($\Delta\delta \approx$ 0.9- to 2.1 ppm) for the carbon at position 2 of benzimidazole ring. All other aliphatic and aromatic protons and carbons were observed at expected regions.

The UV-Vis spectra of free benzimidazoles (**I**−**VII**) and its complexes (**1**−**14**) were determined in 190−800 nm region in DMSO. Free substituted benzimidazoles have absorption maxima at 273,5 nm attributed n- π* transitions. In the complexes, these peaks are shifted to 276.5 nm for the Zn complexes and observed same value (273.5 nm) for the Co complexes. The DMSO-d_6 bands for all cobalt (II) complexes (**2**, **4**, **6**, **8**, **10**, **12** and **14**) were observed as 666.5 nm (ϵ = 219.3 M^{-1} cm^{-1}). All the cobalt (II) complexes showed a single DMSO-d_6 band. Since zinc (II) has no unpaired d-electrons, no absorption peak was observed in the visible region for these complexes.

3.2. Biological activity

3.2.1. Cytotoxicity studies

In order to investigate the cytotoxic effects of newly synthesized cobalt (II) and zinc (II) benzimidazole complexes on A549 and BEAS-2B cells, respective cells were incubated with increasing concentrations (0−100 μg/mL) of compounds for 24, 48 and 72 h, and then subjected to a MTT assay. Table 1 shows the IC$_{50}$ (concentration required to inhibit tumor cell proliferation by 50%) of the compounds. Among the 14 compounds tested, compounds **1**, **4** and **5** had the highest cytotoxic activity while the IC$_{50}$ values of compounds **1**, **4** and **5** were 1.97, 1.87 and 1.9 μg/mL, respectively. This value was 2.56 μg/mL for cisplatin. The compounds **3**, **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14** showed lower cytotoxic activity with IC$_{50}$ values between 9 and 85 μg/mL. The remaining two compounds **2** and **6** showed the least cytotoxic activity with IC$_{50}$ higher than 100 μg/mL (Table 1 and Fig. 1 A, B, C, D). According to the results of 72 h incubation it could be stated that the compound 4 containing 4-bromobenzyl substituent at the position 1 of benzimidazole ligand was more cytotoxic than the compound **1** containing 4-chlorobenzyl and compound **5** containing 4-methylbenzyl substituents on A549 cells. Among the tested 14 complexes, incorporating zinc(II) metal (**1**, **3**, **5**, **7**, **9**, **11** and **13**) showed more cytotoxicity on A549 cells than the other compounds tested except compound **4**. Compound **4** containing cobalt (II) had also high

Table 1
IC$_{50}$ (μg/mL) values of benzimidazole -Co(II) and Zn(II) complexes on A549 cells and BEAS-2B cells.

Compounds	Time (hour)					
	24		48		72	
	A549	BEAS2B	A549	BEAS2B	A549	BEAS2B
1	>100	65,67	27,84	59,77	**1,97**	**59,8**
2	>100	71,69	>100	51,65	>100	42,23
3	>100	62,95	>100	67	13,77	61,59
4	>100	40,54	>100	28,57	**1,87**	**24,5**
5	50,98	37,81	15,02	33,35	**1,9**	**32,67**
6	94,18	83,5	98,52	42,18	>100	1,99
7	74,87	58,85	42,68	53,47	9,36	51,55
8	90,67	39,78	23,94	20,76	13,11	12,00
9	>100	41,22	89,01	38,70	28,55	38,24
10	>100	82,26	>100	62,83	36,74	44,66
11	>100	81,71	82,36	67,91	22,36	60,04
12	>100	>100	76,81	>100	37,82	97,24
13	>100	>100	88,73	>100	77,46	>100
14	>100	91,54	>100	84,31	85,07	57,1
Cisplatin	4,44	3,17	2,72	2,60	**2,56**	**2,53**

Results are averages of twelve independent experiments.
The compounds (**1**,**4** and **5**) showed high cytotoxic activity than cisplatin against A549 whereas the same compounds showed less cytotoxic activityple against BEAS-2B.
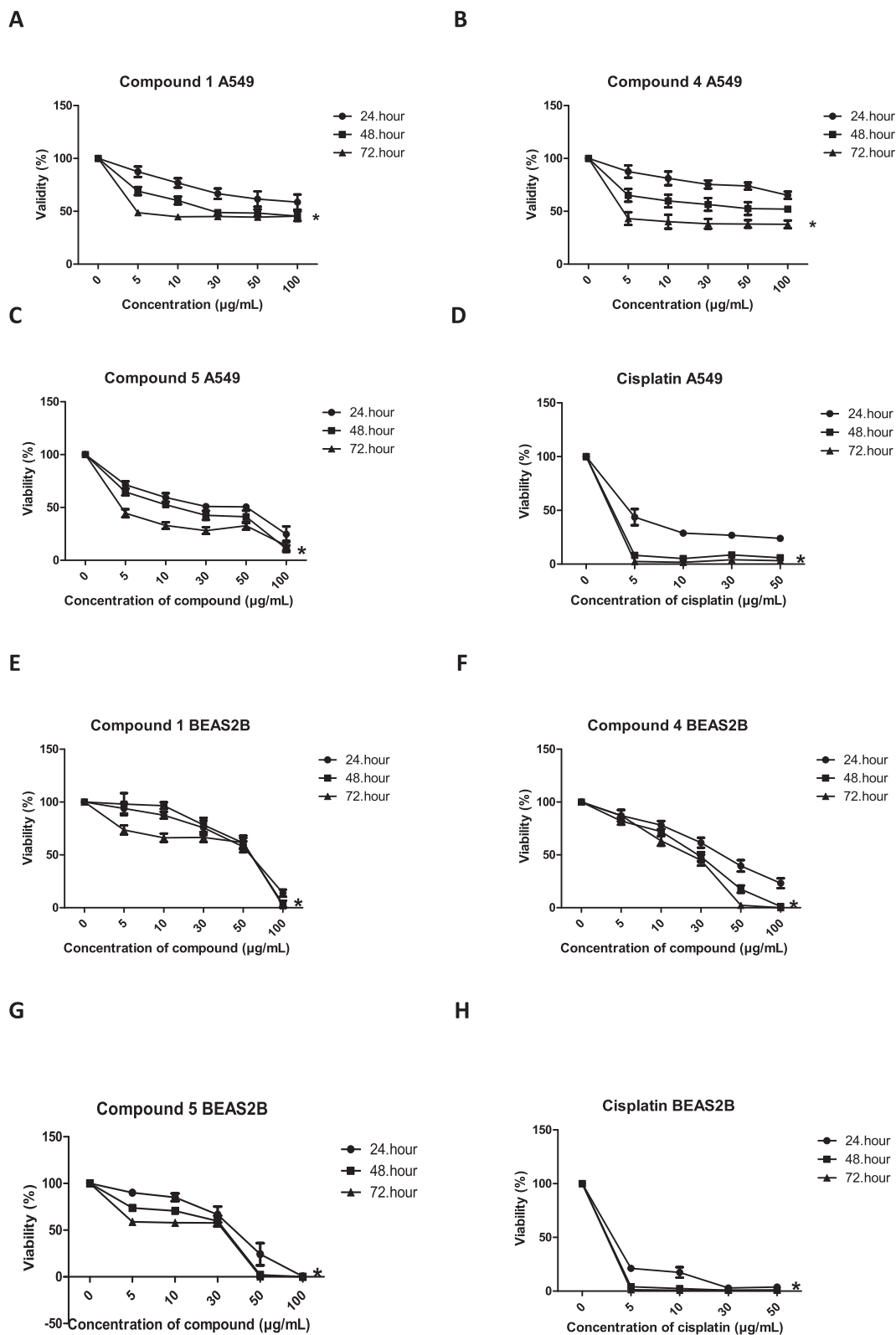
Fig. 1. A, B, C and D. Cytotoxicity activity of compound **1, 4, 5 and cisplatin** on A549, respectively. E, F, G and H. Cytotoxicity activity of compound **1, 4, 5 and cisplatin** on BEAS-2B, respectively. Data points represent means for experiment±SD of twelve independent wells. 72. h significantly different from respective 24 and 48 h: $^{*}p < 0.001$.

cytotoxic activity on A549 cells. Yurttas et al. evaluated the anti-cancer activity of some new1-(2-aryl-2-oxoethyl)-2-[(morpholine-4-yl)thioxomethyl]benzimidazole derivatives on C6, MCF7 and

A549 cell lines and reported the IC_{50} values for 1-(4-chloropheny)-2-(2-(morpholine-4-carbonothioyl)-1H-benzo[d]imidazol-1-yl) ethanone and cisplatin, against A549 cells at 24 h, as 15.66 μg/mL

and 19 μg/mL, respectively [51]. In our study, IC$_{50}$ of the cisplatin was detected as 4.44 μg/mL. Liu et al. suggested that Zn(II) complexes containing bis-benzimidazole derivatives could be candidate for further evaluation as chemotherapeutic agent for human cancers [52]. It was found that one of the new benzimidazole derivative was the most cytotoxic against A549 cells at hypoxic conditions [53].

The cytotoxicity of compounds against human lung bronchial epithelium cells was also examined. IC$_{50}$ values of compounds **1**, **4** and **5** on BEAS-2B were determined as 59.8, 24.5 and 32.67 μg/mL, respectively. This value was 2.53 μg/mL for cisplatin (Table 1 and Fig. 1E, F, G, H). In order to confirm the results, A549 and BEAS-2B cells lines were treated with compounds **1**, **4** and **5** and cisplatin for 72 h at concentration 3 μg/mL. These compounds showed toxic effect against A549 cells as much as cisplatin, but they were less toxic than cisplatin on BEAS-2B (Figs. 2 and 3). Liu et al. evaluated the anticancer activities of two Zinc(II) complexes containing bis-benzimidazole derivatives and cisplatin against five cancer and one normal cell lines. They reported that complex 2 demonstrated higher growth inhibition on MCF-7 human breast carcinoma cells than cisplatin. The complexes possessed high selectivity between human cancer and normal cells (HK-2), in comparison with cisplatin [52].

3.2.2. Antimicrobial studies

The antibacterial and antifungal activities of these newly synthesized compounds were tested by MIC method. Antimicrobial activity results of the complexes were given in Table 2 with standard reference compounds ciprofloxacin and gentamicin. Gram-positive and Gram-negative bacteria have different cell wall structure. While Gram-positive bacteria have a cell wall containing thick peptidoglycan the Gram-negative bacteria have a thin layer of peptidoglycan and also the additional outer membrane. Therefore, this outer membrane is an additional protective barrier against chemicals such as antimicrobials [54,55]. The compounds tested in this work showed antibacterial activity against all the bacteria and the yeast used. All of the compounds showed high antibacterial activity against Gram-positive bacterium *S. aureus* (6.25 μg/mL) but poor antibacterial activity against Gram-negative *P. aeruginosa* (200–400 μg/mL). While the compounds of **1**, **4**, **5**, **13** and **14** showed high antibacterial activity (25–50 μg/mL) against Gram-negative *E. coli*, the others showed moderate activity (100–200 μg/mL) against this bacterium. Among the compounds used **1**, **4** and **5** showed high antibacterial activity against Gram-positive *E. faecium*. These three compounds were also effective antifungal compounds against *C. albicans*. The position and type of the substituent on the benzimidazole ring give different biological
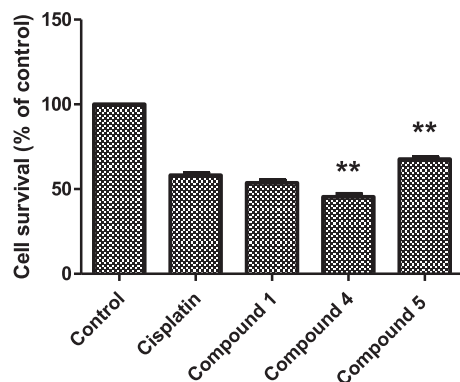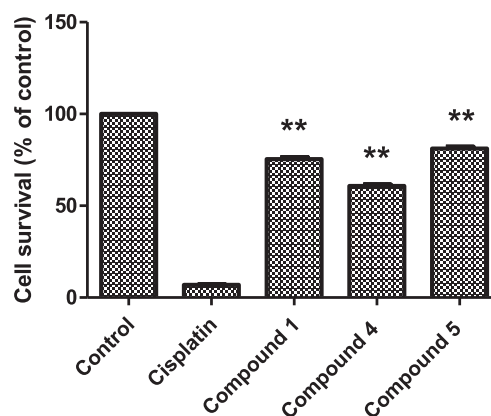
Fig. 3. Cytotoxic effect of cisplatin and compounds **1**, **4** and **5** against BEAS-2B. Compound **1**, **4** and **5** significantly different from respective cisplatin **p < 0.05.

Table 2
In vitro antimicrobial activities MIC data (μg/mL) of benzimidazole -Co(II) and Zn(II) complexes.

Compounds	E. coli	S. aureus	P. aeruginosa	E. faecium	C. albicans
1	25	6,25	200	12,5	6,25
2	200	6,25	400	100	100
3	100	6,25	400	100	50
4	50	6,25	200	50	12,5
5	50	6,25	200	25	12,5
6	100	6,25	400	200	100
7	100	6,25	400	200	100
8	100	6,25	200	200	100
9	200	6,25	400	400	200
10	100	6,25	400	400	200
11	200	6,25	400	200	200
12	100	6,25	400	100	400
13	50	6,25	400	100	400
14	50	6,25	400	200	400
Ciprofloksasin	<0,039	<0,039	<0,039	<0,039	
Gentamisin	<0,024	<0,024	<0,024	<0,024	
Fluconazole					<0,024

activity to the compounds [56]. Some compounds are more effective than the others. The compounds, especially **1**, **4** and **5** which have high antimicrobial activity against all the microorganisms, also showed low cytotoxicity against healthy human lung bronchial epithelium cells (BEAS-2B). Because of their low cytotoxic effects against healthy cells these compounds may be a potential as an antimicrobial agent.

4. Conclusions

The synthesis and cytotoxic activity of fourteen benzimidazole derivatives have been reported in this work. The cytotoxic activities of the new benzimidazole metal complexes on human cancer cells (A549) and normal cells (BEAS-2B) have been evaluated. It can be concluded from our result that compounds **1**, **4** and **5** are more cytotoxic on A549 cells than cisplatin. On the other hand cisplatin has higher cytotoxicity on BEAS-2B cells than the compounds. These compounds are also effective antimicrobial agents. In conclusion, result of this work has encouraged us to investigate their anticancer profiles in further studies.

Declaration of interest

The authors declare no conflicts of interest.

Fig. 2. Cytotoxic effect of cisplatin and compounds **1**, **4** ve **5** against A549. Compound **4** and **5** significantly different from respective cisplatin **p < 0.05.

Acknowledgements

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