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FULL PAPER

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Cytotoxic and antimicrobial potential of benzimidazole derivatives

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Abstract

New benzimidazole derivatives were synthesized and their structures were characterized by spectroscopic and microanalysis techniques. The cytotoxic properties of ten benzimidazole derivatives, five of which were synthesized in our previous studies, were determined against the lung cancer cell line, A549, and the healthy lung epithelial cell line, BEAS-2B. Among the ten compounds tested, based on the 72-h incubation results, compound **12** was the most cytotoxic against the A549 cell line, whereas against the BEAS-2B cell line, it was as cytotoxic as cisplatin. The IC_{50} values of compound **12** were 3.98 and 2.94 μg/ml for A549 and BEAS-2B cells, respectively. The cisplatin values were 6.75 and 2.75 μg/ml for A549 and BEAS-2B cells, respectively. Compounds **10**, **8**, **7**, and **13** showed toxic effects against A549 cells, but were less toxic against BEAS-2B cells than cisplatin. The antimicrobial activity of these compounds against pathogenic bacteria and yeasts was also evaluated based on their minimum inhibitory concentration (MIC) values. The compounds, except **12** and **13**, generally showed higher antimicrobial activity against yeasts, compared with bacteria. Compound **12** showed better activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* than against *Escherichia coli*. Compounds **7**, **8**, and **11** were the most effective ones against the microorganisms, and yeasts were highly sensitive to these compounds with MIC values of 25–100 μg/ml.

KEYWORDS
A549 cells, antimicrobial activity, BEAS-2B cells, benzimidazole derivatives, cytotoxicity

1 | INTRODUCTION

Benzimidazole, which has been studied with interest since the 1800s, is found in the structure of many natural compounds and can be sold commercially as medicine, continues to maintain its privileged place among the heterocyclic compounds. Examples of benzimidazole compounds sold as drugs in pharmacies are albendazole, omeprazole, lansoprazole, pantoprazole, rabeprazole and tenatoprazole, etonitazine, galeterone, mavatrep, and dovitinib.[1–3] The most important natural benzimidazole compound is *N*-riosyldimethylbenzimidazole, which is the ligand of the cobalt atom in vitamin B12.[4] The first synthesis of benzimidazole was the preparation of 2,5 (or 2,6)-dimethylbenzimidazole by Hoebrecker by the reduction of 2-nitro-4-methylacetanilide with Sn/HCl in 1872.[5] Benzimidazole compounds, which have attracted attention since their first synthesis, are being studied with great interest today and continue to maintain their privileged properties. According to the report of the World Health Organization, cancer caused one of every six deaths in 2018 and

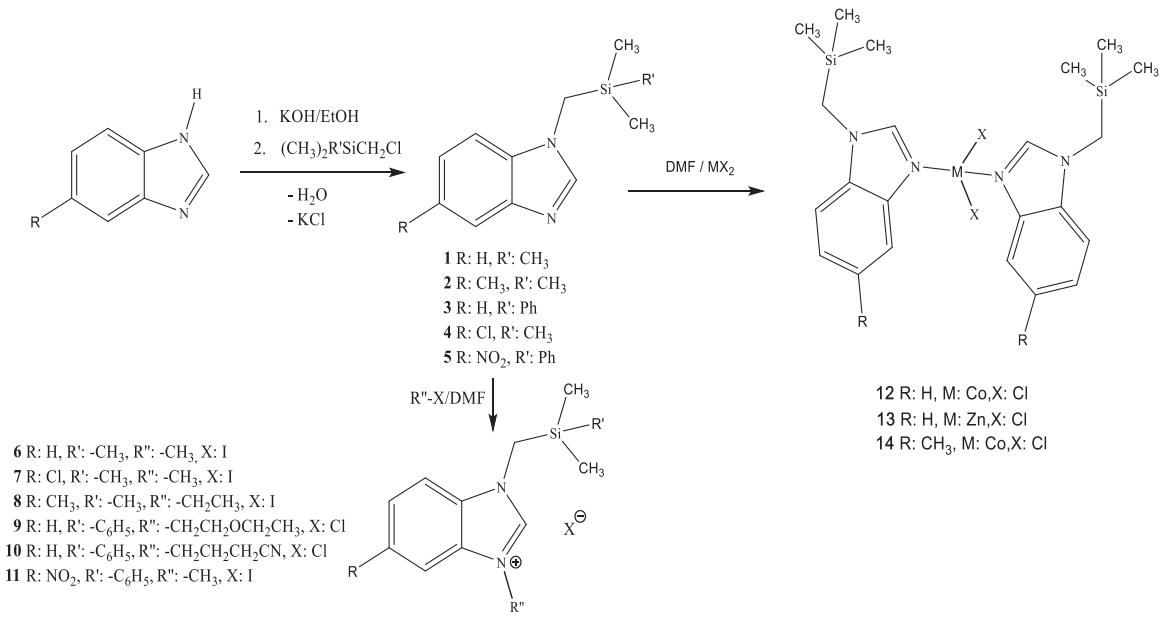
Dedicated to Professor Christian Bruneau.

became the second leading cause of death globally. According to the same report, the most common cancers in men are lung, prostate, colorectal, stomach, and liver cancer, whereas the most common in women are breast, colorectal, lung, cervical, and thyroid cancer. Globally, according to the 2020 report from the International Agency for Research on Cancer, it is estimated that 1 in 5 people develop cancer in their lifetime, and 1 in 8 men and 1 in 11 women die from the disease.[6] Global aging population and socioeconomic risk factors, the ineffectiveness of the drugs used, and the proximity of toxic values to therapeutic values are among the main factors that trigger this increase. Lung cancer is the first cause of cancer deaths worldwide, leading to about 1.6 million deaths per year.[7] Lung cancer is comprised of small cell lung cancer (SCLC) and non-SCLC (NSCLC), among which NSCLC takes up approximately 85% of lung cancer cases.[8] In recent years, significant increases in cancer cases have made the development of anticancer drugs and the synthesis of more active compounds among scientists' primary targets. In the literature, there are notable studies on the anticancer properties of 1-substituted benzimidazole and their 1,3-disubstituted salts and metal complexes such as cobalt and zinc.[9] As we observed significant anticancer activities in some benzimidazole derivatives in our recent studies,[10–12] we examined the anticancer properties of other benzimidazole derivatives in this study. Benzimidazole is an isostere of a purine-based nucleic acid,[13] and benzimidazole derivatives are an important class of heterocyclic compounds with antimicrobial, antioxidant, anticancer, antiproliferative, and antitumor activities.[14–20] Heterocyclic compounds containing heteroatoms such as nitrogen, sulfur, and oxygen have the ability to make strong hydrogen bonding with DNA. The interaction strength between such compounds and DNA is generally associated with anticancer activity.[21,22] Benzimidazole containing two nitrogen atoms is also an important pharmacophore in this respect. In addition to the antitumor properties of benzimidazoles, the presence of a number of clinically approved benzimidazole derivatives such as albendazole, mebendazole, tiabendazole, omeprazole, astemizole, enviradin, candesartan, and telmisartan raises the expectation that the new benzimidazoles will also exhibit potential bioactivities.[23] As microorganisms develop resistance to antibiotics, new antimicrobial agents with antimicrobial activity are needed. For this purpose, the antibacterial and antifungal activities of these benzimidazole derivatives, against various bacteria and yeasts, were also determined.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis pathway utilized to prepare the benzimidazole derivatives **4**, **5**, and **7**–**11** is shown in Scheme 1. The 1-substituted benzimidazole compounds used as starting material were synthesized by refluxing the appropriate benzimidazole with alkyl halide containing silicon in the presence of KOH in ethyl alcohol using Pozharskii and Simonov method.[24] Then, the 1-substituted benzimidazoles prepared were reacted with suitable alkyl halides for 1,3-benzimidazole salts, and cobalt(II) chloride and zinc(II) chloride for cobalt(II) and zinc(II) complexes, respectively. Compounds **1**,[25] **2**,[25] **3**,[26] **6**,[27] **12**,[25] **13**,[25] and **14**[25] published in our previous studies were synthesized again and used after their purity was checked with the data in the literature. Although compounds **4** and **5** are synthesized from benzimidazole, they can be 5- or 6-substituted due to their tautomers.[28] We have verified this situation in our previous studies with the single-crystal X-ray diffraction method. Therefore, when naming these compounds, they



SCHEME 1 Synthesis pathways of the benzimidazole derivatives. DMF, dimethylformamide

were numbered as 5(6)-substituted benzimidazoles.[29,30] When the ^{1}H nuclear magnetic resonance (NMR) spectra of the compounds are examined, the absence of the NH peak observed around 12 ppm in dimethyl sulfoxide (DMSO)-d_6 of the compounds **4** and **5** indicates that the first position of the benzimidazole is alkylated. Although the proton at position 2 of the benzimidazole was observed around 8.10 ppm, it shifted downfield by about 0.70–0.90 ppm due to the electron-withdrawing chlorine and nitro substituents in compounds **4** and **5**. In compounds **7**–**11**, which converted to a salt structure by alkylation of the third position of benzimidazole, the proton at position 2 shifted to the range of 9.48–9.87 ppm. These values show a downfield shift of about 0.7–1.5 ppm, compared with the corresponding 1-substituted benzimidazoles. The peak of carbon at position 2 of benzimidazole salts is generally reported in the range of 142 ± 4 ppm in the literature, and the values in our present study are in agreement with the values in the literature.[31]

2.2 | Pharmacology/biology

2.2.1 | Cytotoxicity studies

Lung adenocarcinoma (A549) and healthy lung bronchial epithelium (BEAS2B) cells were incubated with increasing concentrations (0–100 µg/ml) of the mentioned benzimidazole derivatives for 24, 48, and 72 h with the aim of investigating the cytotoxic effects of the compounds on these cells. After incubation periods (24, 48, and 72 h), cytotoxicity was evaluated colorimetrically by MTT assay. Tables 1 and 2 show the IC_{50} (concentration required to inhibit tumor cell proliferation by 50%) values of the compounds. Among the 10 compounds tested, according to the result of the 72-h incubation, whereas compound **12** is the most cytotoxic against A549 cell line, it has as much cytotoxic effect as cisplatin on BEAS2B cell line. The IC_{50} value of compound **12** was 3.98 µg/ml for A549 and 2.94 µg/ml for BEAS2B. The IC_{50} value of cisplatin was 6.75 µg/ml for A549 and 2.75 µg/ml for BEAS2B. Compounds **10**, **8**, **7**, and **13** showed a toxic effect against A549, but they were less toxic than cisplatin against BEAS-2B. Abdel-Mohsen et al.[32] developed benzimidazole conjugates with pyrimidine and explored their anticancer activities against 12 carcinoma cell lines (KB, SK OV-3, SF-268, NCI H460, RKOP27, HL60, U937, K562, G361, SK-MEL-28, GOTO, NB-1). When the structure–activity relationships (SAR) of the compounds are examined, it is understood that benzimidazole–cobalt complexes **12** and **14** show higher cytotoxicity than other benzimidazole derivatives (Table 1). This effect could possibly be due to the paramagnetic property of cobalt. When the benzimidazole structures showing high cytotoxicity in the second and third ranks are examined, it is seen that the alkyl group at the third position of benzimidazole in compounds **9** and **10** contains oxygen atom and cyano group, which can increase the nucleophilic property, respectively. When Table 2 is examined, it is seen that among compounds **9**, **10**, **12**, and **14** showing high cytotoxic activity, the alkyl structure containing cyano group at the third position of benzimidazole moiety has less effect on healthy lung cell lines (BEAS-2B cell lines).

2.2.2 | Antimicrobial studies

The minimum inhibitory concentration (MIC) test was used to evaluate the antibacterial and antifungal activities of the compounds against bacteria and yeasts, respectively. As shown in Table 3, compound **1** had no antimicrobial activity against the microorganisms. All the compounds showed low antibacterial activity against *Escherichia coli* with MIC values equal or above 800 µg/ml. Therefore, among the bacteria used, *E. coli* was the most resistant bacterium against these compounds. Compounds **7**, **8**, and **11** were the most effective compounds against *E. coli*. However, compound **12** was detected as the most effective compound against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC values of 100 and 200 µg/ml, respectively. Furthermore, the MIC value of compound **7** was also 200 µg/ml against *S. aureus*. Compounds **7**, **8**, and **11** were the most effective compounds against the microorganisms, and yeasts were highly sensitive to these compounds with the MIC values of 25–100 µg/ml. The MIC values of compound **7** for *Candida albicans* and *Candida tropicalis* were in the range of 50–100 µg/ml. However, compounds **8** and **11** with the MIC values of 25 µg/ml showed the highest antifungal activity against these yeasts. When the SAR of compounds **7**, **8**, **11**, and **12** with the highest antimicrobial activity are examined, it is seen that at position 5 of the benzimidazole ring, electron-releasing methyl or electron-withdrawing chlorine and nitro substituents contribute positively to antimicrobial activity, compared with those without substituents (-Table 3, compounds **6**, **9**, and **10**). However, in benzimidazole metal complexes, compound **12** without substituents at the fifth position of the benzimidazole ring exhibited the highest antibacterial activity, whereas its antifungal activity was found to be lower than those without complexes. When the SAR of compounds **12**, **13**, and **14** was examined, cobalt atom contributed more positively to antimicrobial activity than the zinc atom.

3 | CONCLUSION

Of the ten compounds tested, based on the 72-h incubation results, compound **12** was the most cytotoxic against the A549 cell line, whereas in the BEAS2B cell line, it was as cytotoxic as cisplatin. The IC_{50} value of compound **12** was 3.98 and 2.94 µg/ml for A549 and BEAS2B, respectively. Cisplatin values were 6.75 and 2.75 µg/ml for A549 and BEAS2B, respectively. The antimicrobial activity of the compounds was investigated based on MIC values, and compounds **8** and **11** showed the highest antifungal activity against yeasts with an MIC value of 25 µg/ml. Compound **12** showed better activity against *P. aeruginosa* and *S. aureus* than it did against *E. coli*. All of the compounds except compounds **12** and **13** generally showed higher antimicrobial activity against yeasts, compared with bacteria.

TABLE 1 IC_{50} (μg/ml) values of benzimidazole derivatives against A549 cells

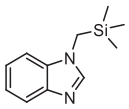
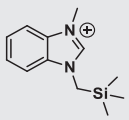
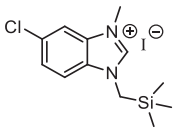
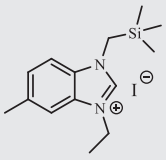
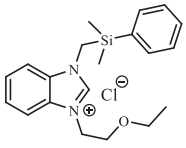
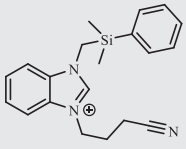
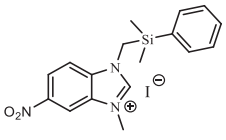
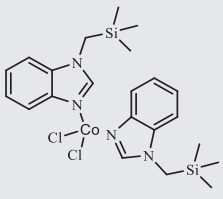
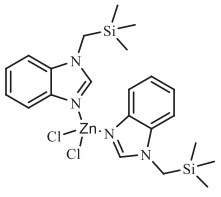
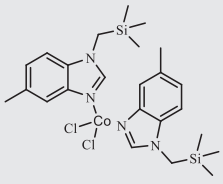
Compounds		Time (h) 24	48	72
1		66.47	51.31	38.11
6		>100	>100	83.40
7		30.83	36.26	38.51
8		65.85	35.49	28.49
9		57.84	37.09	26.22
10		79.03	37.39	24.35
11		71.00	48.46	64.35
12		12.45	4.58	3.98
13		98.11	71.85	60.70
14		46.00	15.12	24.19
Cisplatin		23.55	6.48	6.75

TABLE 2 IC_{50} (μg/ml) values of benzimidazole derivatives against BEAS-2B cells

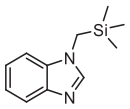
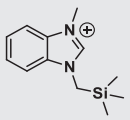
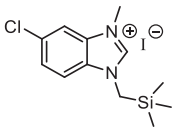
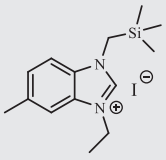
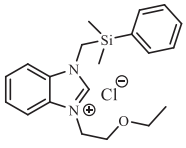
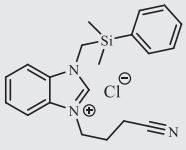
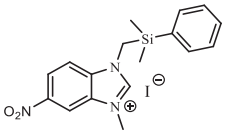
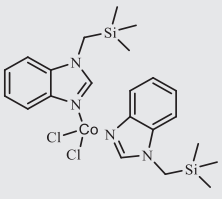
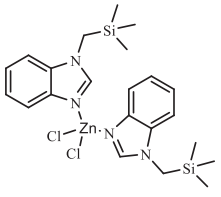
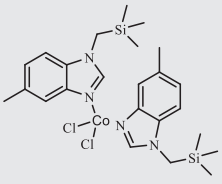
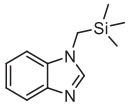
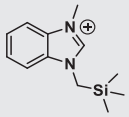
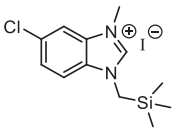
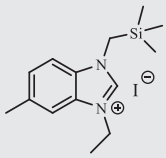
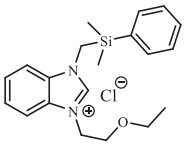
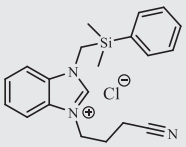
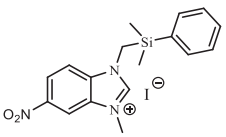
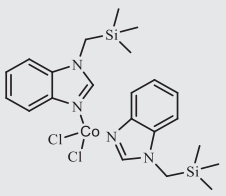
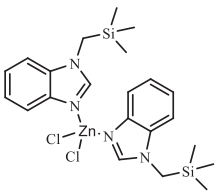
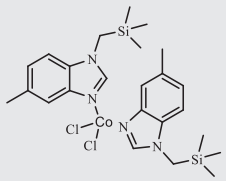
Compounds		Time (h) 24	48	72
1		62.33	49.14	35.79
6		>100	86.35	61.16
7		63.34	61.36	57.89
8		74.13	53.46	43.73
9		37.96	22.66	28.55
10		>100	>100	87.63
11		>100	98.25	13.56
12		4.69	3.17	2.94
13		83.18	83.69	68.53
14		20.21	12.76	9.26
Cisplatin		4.72	2.79	2.75

TABLE 3 MIC values (μg/ml) of benzimidazole complexes

Compounds		*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Candida tropicalis*
1		–	–	–	–	–
6		1600	800	1600	400	400
7		800	400	200	50	100
8		800	400	400	25	25
9		1600	400	400	100	100
10		1600	800	1600	800	800
11		800	400	400	25	25
12		1600	200	100	400	400
13		1600	800	1600	800	800

(Continues)

TABLE 3 (Continued)

Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Candida tropicalis*
14 	1600	800	800	400	800
Gentamicin	0.78	0.39	3.12	–	–
Fluconazole	–	–	–	0.39	0.39

Abbreviation: MIC, minimum inhibitory concentration.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The starting chemicals and solvents used in this study were commercially supplied from Sigma-Aldrich, Acros, Merck, and Tekkim companies. The human lung adenocarcinoma (A549) cancer cell line was provided by Prof. Dr. Fikrettin Sahin (Yeditepe University, Department of Genetics and Bioengineering, Istanbul/Turkey). All bacteria and yeasts used in this study were obtained from stock cultures in the Biotechnology Laboratory, Inonu University. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra (see the Supporting Information) were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Elemental analyses were performed by LECO CHNS-932 elemental analyzer. Infrared spectra were recorded with ATR equipment in the range of 4000–650 cm^{-1} on a PerkinElmer Spectrum one FTIR spectrophotometer. Melting points (mp) were measured in open capillary tubes and were uncorrected, using a Gallenkamp MPD350.BM3.5 apparatus. Compounds **1**,[25] **2**,[25] **3**,[26] **6**,[27] **12**,[25] **13**,[25] and **14**[25] published in our previous studies were synthesized again and used after their purity was checked with the data in the literature.

The InChI codes of the new compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of compounds **1**–**5**[24]

Appropriate alkyl halide (0.01 mol) was added to a mixture of appropriate benzimidazole (0.01 mol) and KOH (0.01 mol) in EtOH (10 ml), and the resulting mixture was refluxed for 4 h. The mixture was then cooled, and potassium halide was filtered off and washed with a little EtOH. The solvent was then removed from the filtrate in vacuum. The residue was washed twice with water (20 ml) and crystallized from EtOH/DMF (2:1) to yield desired compounds **1**–**5**.

4.1.3 | Synthesis of 1-trimethylsilylmethyl-5(6)-chlorobenzimidazole (**4**)

(Chloromethyl)trimethylsilane (0.92 ml, 6.55 mmol) was added to a mixture of 5(6)-chlorobenzimidazole (1.0 g, 6.55 mmol) and KOH (0.37 g, 6.55 mmol) in EtOH (10 ml), and the mixture was heated under reflux for 4 h. The mixture was then cooled, and potassium chloride was filtered off and washed with a little EtOH. The solvent was then removed from the filtrate in vacuum. The residue was washed twice with water (20 ml) and crystallized from EtOH/DMF (2:1). Yellow oily compound **1** was obtained in moderate yield (1.04 g, 66%). M.p.: 231–233°C; $\upsilon_{(CN)}$ = 1577 cm^{-1}. Anal. found: C, 55.18; H, 6.24; N, 11.48%. Calculated for $C_{11}H_{15}N_2ClSi$: C, 55.33; H, 6.33; N, 11.73%. ^{1}H NMR (400 MHz, DMSO) δ 8.80 (s, 1H, NC*H*N), 7.96 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.64 (d, 1H, Ar–*H*, *J* = 8 Hz), 3.97 (s, 2H, CH_2Si), −0.08 (s, 9H, [(CH_3)$_3$Si]). ^{13}C NMR (101 MHz, DMSO) δ 145.3 (NCHN), 142.3, 133.4, 127.4, 122.2, 119.7, and 113.7 (Ar–*C*), 39.3 (CH_2Si) and −0.7 [(CH_3)$_3$Si].

Compound **5** was synthesized according to the method used in the synthesis of compound **4**. M.p.: 128–129°C; $\upsilon_{(CN)}$ = 1579 cm^{-1}. Anal. found: C, 61.68; H, 5.50; N, 13.52%. Calculated for $C_{16}H_{17}N_3O_2Si$: C, 61.71; H, 5.50; N, 13.49%. ^{1}H NMR (400 MHz, DMSO) δ 8.83 (s, 1H, NC*H*N), 7.40 (s, 1H, Ar–*H*), 8.21 (d, 1H, Ar–*H*, *J* = 8 Hz), 8.13 (d, 1H, Ar–*H*, *J* = 8.0 Hz), 7.54–7.28 (m, 5H, Ar–*H*), 4.30 (s, 2H, CH_2Si), 0.35 (s, 6H, [(CH_3)$_2$SiPh]). ^{13}C NMR (101 MHz, DMSO) δ 146.7 (NCHN), 144.9, 134.7, 133.9, 131.6, 128.0, 127.6, 122.9, 121.6, 115.1, 110.1 (Ar–*C*), 38.1 (CH_2Si), −3.9 [(CH_3)$_2$SiPh].

4.1.4 | General procedure for the synthesis of compounds **6**–**11**

A mixture of an appropriate 1-substituted benzimidazole (0.010 mol) and an appropriate alkyl halide (0.011 mol) in dimethylformamide (DMF) (5 ml) was heated under reflux for 3 h. The mixture was then cooled and the volatiles were removed under vacuum. The residue was crystallized from a DMF/ethanol mixture (1:1) to yield target compounds **6**–**11**.

4.1.5 | Synthesis of 5-chloro-3-methyl-1-[(trimethylsilyl)methyl]-2,3-dihydro-1*H*-benzo[*d*]-imidazolium iodide (**7**)

A mixture of 1-trimethylsilylmethyl-5(6)-chlorobenzimidazole (1.1 g, 4.60 mmol) and iodomethane (0.30 cm^3, 4.80 mmol) in DMF (5 ml) was heated under reflux for 3 h. The mixture was then cooled and the volatiles were removed under vacuum. The residue was crystallized from a DMF/ethanol mixture (1:1). White crystals of the title compound **7** (1.34 g, 76%) were obtained, m.p. 201–202°C; υ(CN) = 1559 cm^{-1}. Anal. found: C, 37.34; H, 4.53; N, 7.05%. Calculated for $C_{12}H_{18}N_2ClISi$: C, 37.86; H, 4.77; N, 7.36%. ^{1}H NMR (400 MHz, DMSO) δ 9.49 (s, 1H, NCHN), 8.17 (s, 1H, Ar–H, 8.02 (d, 1H, Ar–H, *J* = 8.0 Hz), 7.64 (d, 1H, Ar–H, *J* = 8.0 Hz), 4.11 (s, 2H, CH_2Si), 3.96 (s, 3H, CH_3), −0.00 (s, 9H, [$(CH_3)_3Si$]). ^{13}C NMR (101 MHz, DMSO) δ 146.5 (NCHN), 144.9, 134.7, 133.0, 129.0, 117.8, 115.8 (Ar–C), 40.00 (CH_2Si), 35.73 (CH_3), −0.44 [$(CH_3)_3Si$].

Compounds **8**, **9**, **10**, and **11** were synthesized according to the method used in the synthesis of compound **7**.

*3-Ethyl-5-methyl-1-[(trimethylsilyl)methyl]-1H-benzo[d]imidazol-3-ium iodide (**8**)*

M.p.: 97–98°C; $\upsilon_{(CN)}$ = 1576 cm^{-1}. Anal. found: C, 67.92; H, 9.37; N, 11.44%. Calculated for $C_{14}H_{23}N_2ISi$: C, 67.96; H, 9.37; N, 11.32%. ^{1}H NMR (400 MHz, DMSO) δ 9.48 (s, 1H, NC*H*N), 7.85 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.79 (s, 1H, Ar–*H*), 7.41 (d, 1H, Ar–*H*, *J* = 8 Hz), 4.41 (q, 2H, CH_2CH_3, *J* = 8 Hz), 4.06 (s, 2H, CH_2Si), 2.43 (s, 3H, CH_3), 1.41 (t, 3H, CH_2CH_3, *J* = 8 Hz), 0.00 (s, 9H, [$(CH_3)_3Si$]). ^{13}C NMR (101 MHz, DMSO) δ 142.3 (NCHN), 138.6, 134.3, 131.1, 130.1, 115.3, 115.0 (Ar–C), 44.1 (CH_2CH_3), 39.9 (CH_2Si), 23.4 (CH_3), 16.7 (CH_2CH_3), −0.50 [$(CH_3)_3Si$].

*1-{[Dimethyl(phenyl)silyl]methyl}-3-(2-ethoxyethyl)-1H-benzo[d]-imidazol-3-ium chloride (**9**)*

M.p.: 121–122°C; $\upsilon_{(C=N)}$ = 1590 cm^{-1}. Anal. found: C, 70.69; H, 8.01; N, 8.29%. Calculated for $C_{20}H_{27}N_2OSi$: C, 70.75; H, 8.02; N, 8.25%. ^{1}H NMR (400 MHz, DMSO) δ 9.73 (s, 1H, NC*H*N), 8.07 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.88 (d, 1H, Ar–*H*, *J* = 8.0 Hz), 7.62 (d, 1H, Ar–*H*, *J* = 8.0 Hz), 7.56–7.50 (m, 6H, Ar-*H*), 4.70 (q, 2H, CH_2CH_3, *J* = 4 Hz), 4.51 (s, 2H, CH_2Si), 3.78 (t, 2H, OCH_2, *J* = 4 Hz), 3.43 (t, 2H, NCH_2, *J* = 4 Hz), 1.03 (t, 3H, CH_2CH_3, *J* = 4 Hz), −0.39 (s, 6H, [(CH_3)2PhSi]). ^{13}C NMR (101 MHz, DMSO) δ 141.9 (NCHN), 134.8, 134.1, 131.9, 131.4, 130.4, 128.4, 126.9, 126.5, 114.4, 114.1 (Ar–C), 66.1 (CH_2CH_3), 47.1(CH_2Si), 37.9 (CH_2CH_2O), 34.2 (CH_2CH_2O), 15.6 (CH_3), −4.0 [(CH_3)2PhSi].

*3-(3-Cyanopropyl)-1-{[dimethyl(phenyl)silyl]methyl}-1H-benzo[d]-imidazol-3-ium chloride (**10**)*

M.p.: 108–109°C; $\upsilon_{(C\equiv N)}$ = 2200 cm^{-1}, $\upsilon_{(C=N)}$ = 1615 cm^{-1}. Anal. found: C, 71.73; H, 7.22; N, 12.62%. Calculated for $C_{20}H_{24}N_3ClSi$: C, 71.81; H, 7.23; N, 12.56%. ^{1}H NMR (400 MHz, DMSO) δ 9.87 (s, 1H, NC*H*N), 8.11 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.94 (d, 1H, Ar–*H*, *J* = 8.0 Hz), 7.67–7.54 (m, 4H, Ar–*H*), 7.53–7.33 (m, 3H, Ar–*H*), 4.61 (t, 2H, CH_2CN, *J* = 6 Hz), 4.46 (s, 2H, CH_2Si), 2.65 (t, 2H, CH_2N, *J* = 8 Hz), 2.19 (t, 2H, $CH_2CH_2CH_2$, *J* = 6 Hz), −0.43 (s, 6H, [$(CH_3)_2SiPh$]). ^{13}C NMR (101 MHz, DMSO) δ 148.1 (NCHN), 134.8, 134.4, 132.2, 131.4, 130.5, 128.4, 126.9, 126.6, 120.3, 114.5 (Ar–C), 113.9 (NC), 45.8 ($CNCH_2$), 38.0 (CH_2Si), 25.2 (CH_2N), 14.1 (CH_2CH_2)CH_2, −3.7 [$(CH_3)_2SiPh$].

*1-{[Dimethyl(phenyl)silyl]methyl}-3-methyl-5-nitro-1H-benzo[d]-imidazol-3-ium iodide (**11**)*

M.p.: 169–170°C; $\upsilon_{(C=N)}$ = 1630 cm^{-1}. Anal. found: C, 62.48; H, 6.18; N, 12.76. Calculated for $C_{17}H_{20}IN_3O_2Si$: C, 62.55; H, 6.18; N, 12.87. ^{1}H NMR (400 MHz, DMSO) δ 9.74 (s, 1H, NC*H*N), 9.04 (s, 1H, Ar–*H*), 8.41 (d, 1H, Ar–*H*, *J* = 8 Hz), 8.13 (d, 1H, Ar–*H*, *J* = 8 Hz), 7.94 (d, 1H, Ar–*H*, *J* = 8.0 Hz), 7.55–7.26 (m, 5H, Ar–*H*), 4.50 (s, 2H, CH_2Si), 4.16 (s, 3H, NCH_3), 0.42 (s, 6H, [$(CH_3)_2SiPh$]. ^{13}C NMR (101 MHz, DMSO) δ 147.8 (NCHN), 145.5, 135.3, 134.1, 131.5, 130.4, 128.5, 128.4, 120.9, 115.5, 110.9 (Ar–C), 38.6 (CH_2Si), 34.5 (NCH_3), −3.8 [$(CH_3)_2SiPh$].

4.1.6 | General procedure for the synthesis of compounds **12**–**14**[25]

A solution of an appropriate 1-substituted benzimidazole (0.02 mol) and cobalt or zinc(II) chloride (0.01 mol) in DMF (5 ml) was heated under reflux for 2 h. The mixture was then cooled to room temperature, and then the solvent was removed from the filtrate in vacuo. The resulting precipitate was then crystallized from EtOH/DMF (2:1).

4.2 | Biological assays

4.2.1 | Cytotoxicity study

In this study, the human lung adenocarcinoma (A549) provided by Prof. Dr. Fikrettin Sahin (Yeditepe University, Department of Genetics and Bioengineering, Istanbul/Turkey) and healthy human lung bronchial epithelium (BEAS-2B) cancer cell lines were used. The cells were maintained in Dulbecco's modified Eagle's medium growth medium containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% CO_2. The cells were seeded at 5×10^3 cells/well in a 96-well plate. After 24 h, cells were exposed to the compounds (range concentration, 0–100 μg/ml) prepared in DMSO (in the final culture medium was <0.1%) and the cells were incubated for 24, 48, and 72 h. The cells were, then, treated with 20 μL of MTT for 4 h at 30°C to evaluate cytotoxic activity of the analyzed compounds. The purple formazan crystals formed by alive cells were solubilized in DMSO solution and optical density was measured at 570 nm. For each concentration, 12 wells were used and IC_{50} values (μg/ml) were defined as the compound concentrations that reduced the absorbance by 50% with respect to control values. Cisplatin was also used as a control agent.

4.2.2 | Antimicrobial study

Bacteria *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853, and yeasts *C. albicans* and *C. tropicalis* were used to test

the antimicrobial activity. These bacteria and yeasts are stock cultures in the Biotechnology Laboratory, Inonu University.

In this study, MIC values of the studied compounds were investigated to detect their antibacterial and antifungal activities, and thus to determine the sensitivity of the microorganisms. All the compounds used were prepared by dissolving them in DMSO, and their serial dilutions were made in sterile 96-well microplates. The cultures of the microorganisms were prepared by growing bacteria and yeasts in Mueller–Hinton agar and Sabouraud dextrose agar media, respectively. Pure colonies from these cultures were used to prepare the cell suspensions based on the McFarland standard. An appropriate amount from these suspensions was inoculated into each well. Sterility control and growth control wells were also used. The plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi, respectively. The lowest concentration of each compound with no growth was recorded as the MIC value. Gentamicin was used as a positive control against bacteria and fluconazole for yeasts.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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REFERENCES

[1] K. Kamanna, *Chemistry and Applications of Benzimidazole and its Derivatives* (Ed: M. Marinescu), IntechOpen **2019**. Ch. 4. pp. 1–19. https://doi.org/10.5772/intechopen.85229
[2] D. S. Son, E. S. Lee, S. E. Adunyah, *Immune Network* **2020**, *20*, 1. https://doi.org/10.4110/in.2020.20.e29
[3] L. Olbe, E. Carlsson, P. Lindberg, *Nat. Rev. Drug. Discov.* **2003**, *2*, 132. https://doi.org/10.1038/nrd1010
[4] S. M. Shaharyar, A. Mazumder, *Arab. J. Chem* **2017**, *10*, S157. https://doi.org/10.1016/j.arabjc.2012.07.017
[5] J. B. Wright, *Chem. Rev.* **1951**, *48*, 397. https://doi.org/10.1021/cr60151a002
[6] GLOBOCAN 2020: New Global Cancer Data, **2020**. https://www.uicc.org/news/globocan-2020-new-global-cancer-data#:~:text=IARC%20released%20on%2014th%20December,million%20cancer%20deaths%20in%2020
[7] G. Tiansheng, H. Junming, W. Xiaoyun, C. Peixi, D. Shaoshan, C. Qianping, *Cell J.* **2020**, *22*, 375. https://doi.org/10.22074/cellj.2020.6837
[8] X. Zhou, Z. Zhang, X. Liang, *Cell J.* **2020**, *21*, 459. https://doi.org/10.22074/cellj.2020.6281
[9] E. Lukevics, P. Arsenyan, I. Shestakova, I. Domracheva, A. Nesterova, O. Pudova, *Eur. J. Med. Chem.* **2001**, *36*, 507. https://doi.org/10.1016/S0223-5234(01)01241-7
[10] Ü. Yılmaz, S. Tekin, N. Buğday, K. Yavuz, H. Küçükbay, S. Sandal, *Inorganica. Chim. Acta* **2019**, *495*, 1. https://doi.org/10.1016/j.ica.2019.118977
[11] H. Küçükbay, A. Mumcu, S. Tekin, S. Sandal, *Turkish J. Chem.* **2016**, *40*, 393. https://doi.org/10.3906/kim-1510-15
[12] E. Apohan, Ü. Yilmaz, Ö. Yilmaz, A. Serindağ, H. Küçükbay, Ö. Yesilada, Y. Baran, *J. Organomet. Chem.* **2017**, *828*, 52. https://doi.org/10.1016/j.jorganchem.2016.11.020
[13] I. Ali, M. N. Lone, H. Y. Aboul-Enein, *MedChemComm* **2017**, *8*, 1742. https://doi.org/10.1039/c7md00067g
[14] E. Taherian, G. Khodarahmi, M. R. Khajouei, F. Hassanzadeh, N. Dana, *Res. Pharm. Sci.* **2019**, *14*, 247. https://doi.org/10.4103/1735-5362.258493
[15] Y. Bansal, O. Silakari, *Bioorg. Med. Chem.* **2012**, *20*, 6208. https://doi.org/10.1016/j.bmc.2012.09.013
[16] İ. Yildiz-Oren, İ. Yalcin, E. Aki-Şener, N. Uçartürk, *Eur. J. Med. Chem.* **2004**, *39*, 291. https://doi.org/10.1016/j.ejmech.2003.11.014
[17] A. Baldisserotto, M. Demurtas, I. Lampronti, M. Tacchini, D. Moi, G. Balboni, S. Pacifico, S. Vertuani, S. Manfredini, V. Onnis, *Bioorg. Chem.* **2020**, *94*, 103396. https://doi.org/10.1016/j.bioorg.2019.103396
[18] H. Küçükbay, R. Durmaz, N. Okyucu, S. Günal, *Folia Microbiol.* **2003**, *48*, 679.
[19] B. Caymaz, U. Yıldız, S. Akkoç, Z. Gerçek, A. Şengül, B. Coban, *ChemistrySelect* **2020**, *5*, 8465. https://doi.org/10.1002/slct.202001580
[20] B. Çetinkaya, E. Çetinkaya, H. Küçükbay, R. Durmaz, *Drug Res.* **1996**, *33*, 821.
[21] J. Akhtar, A. A. Khan, Z. Ali, R. Haider, M. S. Yar, *Eur. J. Med. Chem.* **2017**, *125*, 143. https://doi.org/10.1016/j.ejmech.2016.09.023
[22] Y. Özkay, I. Işikdağ, Z. İncesu, G. Akalın, *Eur. J. Med. Chem.* **2010**, *45*, 3320. https://doi.org/10.1016/j.ejmech.2010.04.015
[23] Ş. Demirayak, I. Kayagil, L. Yurttaş, *Eur. J. Med. Chem.* **2011**, *46*, 411. https://doi.org/10.1016/j.ejmech.2010.11.007
[24] A. F. Pozharskii, A. M. Simonov, *Russ. J. Gen. Chem.* **1963**, *33*, 172.
[25] N. Şireci, H. Küçükbay, M. Akkurt, Ş. P. Yalçin, M. N. Tahir, H. Ott, *J. Coord. Chem.* **2010**, *63*, 3218. https://doi.org/10.1080/00958972.2010.509432
[26] H. Küçükbay, N. Şireci, Ü. Yilmaz, S. Deniz, M. Akkurt, Z. Baktir, O. Büyükgüngör, *Turkish J. Chem.* **2012**, *36*, 201. https://doi.org/10.3906/kim-1109-5
[27] Ü. Yılmaz, H. Küçükbay, N. Şireci, M. Akkurt, S. Günal, R. Durmaz, M. N. Tahir, *Appl. Organomet. Chem.* **2011**, *25*, 366. https://doi.org/10.1002/aoc.1772
[28] A. Kaiser, *Chem. Ber.* **1885**, *18*, 2942.
[29] S. Öztürk, M. Akkurt, H. Kücükbay, N. Okuyucu, H. K. Fun, *Acta Crystallogr., Sect. E: Crystallogr. Commun.* **2003**, *E59*, o1014. https://doi.org/10.1107/S1600536803013473
[30] S. Ö. Yıldırım, M. Akkurt, H. Küçükbay, E. Orhan, O. Büyükgüngör, *Acta Crystallogr., Sect. E: Crystallogr. Commun.* **2005**, *61*, 2038. https://doi.org/10.1107/S1600536805017502
[31] H. Küçükbay, N. Şireci, Ü. Yilmaz, M. Akkurt, Ş. P. Yalçin, M. N. Tahir, H. Ott, *Appl. Organomet. Chem.* **2011**, *25*, 255. https://doi.org/10.1002/aoc.1751
[32] H. T. Abdel-Mohsen, F. A. F. Ragab, M. M. Ramla, H. I. El Diwani, *Eur. J. Med. Chem.* **2010**, *45*, 2336. https://doi.org/10.1016/j.ejmech.2010.02.011

SUPPORTING INFORMATION

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