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Synthesis and Antimicrobial Activities of Some Transition Metal Benzimidazole Complexes

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The interaction of N-monosubstituted benzimidazoles with various metal salts [$CoCl_2$, $NiCl_2$ and $Zn(OAc)_2$)] were yielded stable solid metal benzimidazole complexes. All the metal complexes were identified by ^1H NMR, FT-IR spectroscopic techniques, elemental analysis and screened for their *in vitro* antimicrobial activities against the standard strains: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and yeasts *Candida albicans* and *Candida tropicalis*. Some of the compounds inhibited the growth of gram-positive bacteria (*E. faecalis* and *S. aureus*) at MIC values between 200 and 800 µg/mL. None of the compounds exhibit antimicrobial activity against gram-negative bacteria (*E. coli* and *P. aeruginosa*) at the concentrations studied (6.25-800 µg/mL). Some of the tested compounds exhibit an antifungal activity with a range of the MICs between 100 and 800 µg/mL.

Key Words: Benzimidazole derivatives, Benzimidazole metal complexes, Antibacterial activity, Antifungal activity.

INTRODUCTION

Benzimidazole derivatives constitute an important class of heterocyclic compounds for their versatile pharmacological activities such as antibacterial, antifungal, antihelmintic, antiallergic, local analgesic, antihistaminic, hypotensive and spalmolytic activities[1-11]. Benzimidazole analogues can be suitably modified by the introduction of different heterocyclic moieties to exhibit a broad spectrum of biological activities such as potent antibacterial, antifungal, anticancerous drugs[12]. When the ligands coordinate to the transition metals, it is believed that they react selectively toward certain biological systems and sometimes even more effective than the free ligand[13].

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The benzimidazole ring is an important pharmacophore in drug discovery. Since the use of cis-platin [$Pt(NH_3)_2Cl_2$)] as an effective anticancer drug, the interest toward transition metal complexes containing N-donor ligands has increased in order to obtain metal based drugs exhibiting a high biological activity together with a reduced toxicity[14-16]. In this respect benzimidazole derivatives and their transition metal complexes have been extensively investigated. One of the most attractive features of these ligands in the field of biological investigation has been their structural similarities with the common pyrimidine and purine type nucleobases[17-19]. Copper(II) and silver(I) complexes of 2-pyridyl-1*H*-benzimidazoles possess considerable activity against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri* and *Candida albicans*[20]. Similar to the literature reports, we also observed that N-monosubstituted benzimidazole, benzimidazolium salts, electron rich olefins derived benzimidazole compounds, organic and organometallic derivatives of benzimidazoles and *bis*-benzimidazoles have shown considerable *in vitro* antimicrobial activities against the standard strains: *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and yeasts *Candida albicans* and *Candida tropicalis*[3-11].

The aim of this study is to synthesize novel metal complexes of 1-substituted benzimidazole compounds and to explore their antibacterial and antifungal activities.

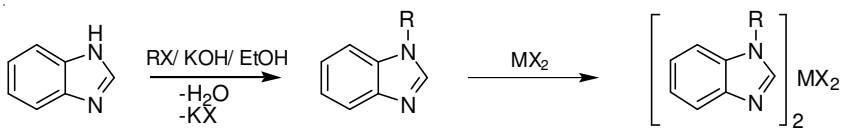
EXPERIMENTAL

All preparations were carried out in an atmosphere of air using standard Schlenk techniques. Starting materials and reagents used in reactions were supplied commercially from Aldrich or Merck Chemical Co. Solvents were dried according to standard methods and freshly distilled prior to use. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using a Bruker DPX-300 high performance digital FT NMR spectrometer. Infrared spectra were recorded as KBr pellets in the range 4000-400 cm^{-1} on a Perkin-Elmer FT-IR 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-Substitutedbenzimidazoles (**1-5**)[3,8], the complexes **6**[21] and **7**[22] were prepared according to the reported method.

Synthesis

General procedure for the preparation of metal benzimidazole complexes: Bivalent metal salt hydrates (2.5 mmol) and 1-substituted benzimidazole ligand (5 mmol) in 25 mL of ethanol in a reaction flask were heated under reflux for 4 h. The mixture was then allowed to stand at room temperature overnight to give a solid product. This was then filtered and the crude product obtained was crystallized from ethanol/2-propanol (3:1). The synthesized compounds are given in **Scheme-I**.



1 R= CH_2CH_2OEt
2 R= CH_2CH_2OMe
3 R= CH_2CH_2Ph
4 R= $CH_2CHOCH{=}CH_2$
5 R= $CH_2CH_2CH_2CN$

6	R = CH_2CH_2OEt	M = Co	X = Cl
7	R = CH_2CH_2OEt	M = Ni	X = Cl
8	R = CH_2CH_2OEt	M = Zn	X = Cl
9	R = CH_2CH_2OMe	M = Co	X = Cl
10	R = CH_2CH_2OMe	M = Ni	X = Cl
11	R = CH_2CH_2OMe	M = Zn	X = Cl
12	R = CH_2CH_2Ph	M = Co	X = Cl
13	R = CH_2CH_2Ph	M = Ni	X = Cl
14	R = CH_2CH_2Ph	M = Zn	X = Cl
15	R = $CH_2CH_2OCH{=}CH_2$	M = Co	X = Cl
16	R = $CH_2CH_2OCH{=}CH_2$	M = Ni	X = Cl
17	R = $CH_2CH_2CH_2CN$	M = Co	X = Cl
18	R = $CH_2CH_2CH_2CN$	M = Ni	X = Cl

Scheme-I: Synthesis pathways of the novel benzimidazole metal complexes **8-18**

Dichloro*bis*[1-(2-ethoxyethyl)-1*H*-benzimidazole-$_K$N^3]nickel(II) (8): Yield: 70 %; m.p. > 300 °C; Anal. calcd. (%) for $C_{22}H_{28}N_4O_2ZnCl_2$: C, 52,71; H, 6.27; N, 10.25. Found (%): C, 52,27; H, 6.18; N, 9.86. IR (KBr, cm^{-1}): 1546 (C=N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.3 (t, CH_3CH_2O-, 6H), 3.3 (q, CH_3CH_2O-4H), 3.8 (t, NCH_2CH_2O-, 4H), 3.6 (t, NCH_2CH_2O-, 4H), 7.5-8.1 (m, Ar-H, 8H), 8.5 (s, CH, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 144.3, 133.2, 122.98, 122.46, 121.82, 119.13, 110. 94, 68,42, 58.64, 58.72, 44.93.

Dichloro*bis*[1-(2-methoxyethyl)-1*H*-benzimidazole-$_K$N^3]cobalt(II) (9): Yield: 84 %; m.p. 170-171 °C; Anal. calcd. (%) for $C_{20}H_{24}N_4O_2CoCl_2$: C, 49,81; H, 5.02; N, 11.62. Found (%): C, 49,73; H, 5.00; N, 11.63; IR (KBr, cm^{-1}): 1514 (C=N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.3 (s, CH_3, 6H), 3.9 (t, NCH_2CH_2O-, 4H), 3.9 (t, NCH_2CH_2O-, 4H), 7.4-8.2 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Dichloro*bis*[1-(2-methoxyethyl)-1*H*-benzimidazole-$_K$N^3]nickel(II) (10): Yield: 78 %; m.p. 96-97 °C; Anal. calcd. (%) for $C_{20}H_{24}N_4O_2NiCl_2$: C, 49,83; H, 5.02; N, 11.62. Found (%): C, 49,42; H, 5.01; N, 11.27; IR (KBr, cm^{-1}): 1512 (C=N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.3 (s, CH_3, 6H), 3.3 (t, NCH_2CH_2O-, 4H), 3.8 (t, NCH_2CH_2O-, 4H), 7.4-8.2 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Dichloro*bis*[1-(2-methoxyethyl)-1*H*-benzimidazole-$_K$N^3]zinc(II) (11): Yield: 72 %, m.p. > 340 °C; Anal. calcd. (%) for $C_{20}H_{24}N_4O_2ZnCl_2$: C, 50,93; H, 5.83; N, 10.80. Found (%): C, 50,79; H, 5.76; N, 10.62; IR (KBr, cm^{-1}): 1547 (C=N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.4 (s, CH_3, 6H), 3.7 (t, NCH_2CH_2O-, 4H), 4,0 (t, NCH_2CH_2O-, 4H), 7.2-7.9 (m, Ar-H, 8H), 8.3 (s, CH, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 145.3, 133.9, 123.48, 122.86, 122.84, 119.23, 111.64, 70,52, 58.52, 44.88.

Dichloro*bis*[1-(3-phenylethyl)-1*H*-benzimidazole-$_K$N^3]cobalt(II) (12): Yield: 72 %; m.p. 203-204 °C; Anal. calcd. (%) for $C_{30}H_{28}N_4CoCl_2$: C, 62,73; H, 4.91; N,

9.75. Found (%): C, 62,07; H, 4.89; N, 9.62; IR (KBr, cm^{-1}): 1523 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.1 (t, NCH_2CH_2Ph-, 4H), 4.8 (t, NCH_2CH_2Ph-, 4H), 7.3-8.1 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Dichloro*bis*[1-(3-phenylethyl)-1*H*-benzimidazole-$_K$N^3]nickel(II) (13): Yield: 79 %; m.p. 155-156 °C; Anal. calcd. (%) for $C_{30}H_{28}N_4NiCl_2$: C, 62,76; H, 4.92; N, 9.76. Found (%): C, 61,97; H, 4.90; N, 9.52; IR (KBr, cm^{-1}): 1521 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.2 (t, NCH_2CH_2Ph-, 4H), 4.8 (t, NCH_2CH_2Ph-, 4H), 7.3-8.1 (m, Ar-H, 8H), 8.5 (s, CH, 2H).

Dichloro*bis*[1-(3-phenylethyl)-1*H*-benzimidazole-$_K$N^3]zinc(II) (14): Yield: 69 %; m.p. > 340 °C; Anal. calcd. (%) for $C_{30}H_{28}N_4ZnCl_2$: C, 62,91; H, 5.61; N, 9.17. Found (%): C, 61,78; H, 5.56; N, 9.07; IR (KBr, cm^{-1}): 1547 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.1 (t, NCH_2CH_2Ph-, 4H), 4.6 (t, NCH_2CH_2Ph-, 4H), 7.3-8.2 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Dichloro*bis*[1-(3-ethoxyvinyl)-1*H*-benzimidazole-$_K$N^3]cobalt(II) (15): Yield: 71 %; m.p. 141-142 °C; Anal. calcd. (%) for $C_{22}H_{24}N_4O_2CoCl_2$ C, 52,19; H, 4.78; N, 11.07. Found (%): C, 52,13; H, 4.77; N, 1.02; IR (KBr, cm^{-1}): 1536 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.2 (d, CH_2=CH-O-, 4H), 4.7 (t, CH_2=CH-O-, 4H), 4.2 (t, NCH_2CH_2-O-, 4H), 4.8 (t, NCH_2CH_2-O-, 4H), 7.6-8.1 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Dichloro*bis*[1-(3-ethoxyvinyl)-1*H*-benzimidazole-$_K$N^3]nickel(II) (16): Yield: 80 %; m.p. 114-115 °C; Anal. calcd. (%) for $C_{22}H_{24}N_4O_2NiCl_2$ C, 52,22; H, 4.78; N, 11.07. Found (%): C, 51.98; H, 4.76; N, 10.87; IR (KBr, cm^{-1}): 1516 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.1 (d, CH_2=CH-O-, 4H), 4.5 (t, CH_2=CH-O-, 4H), 4.2 (t, NCH_2CH_2-O-, 4H), 4.7 (t, NCH_2CH_2-O-, 4H), 7.6-8.1 (m, Ar-H, 8H), 8.2 (s, CH, 2H).

Dichloro*bis*[1-(3-cyanopropyl)-1*H*-benzimidazole-$_K$N^3]cobalt (II) (17): Yield: 75 %; m.p. 287-288 °C; Anal. calcd. (%) for $C_{22}H_{22}N_6CoCl_2$ C, 52,82; H, 4.43; N, 16.80. Found (%): C, 52,73; H, 4.43; N, 16.62; IR (KBr, cm^{-1}): 1523 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.2 (m, $CH_2CH_2CH_2$-CN, 4H), 2.5 (t, $CH_2CH_2CH_2$-CN, 4H), 3.9 (t, $CH_2CH_2CH_2$-CN, 4H), 7.9-8.1 (m, Ar-H, 8H), 8.5 (s, CH, 2H).

Dichloro*bis*[1-(3-cyanopropyl)-1*H*-benzimidazole-$_K$N^3]nickel(II) (18): Yield: 73 %; m.p. 278-279 °C; Anal. calcd. (%) for $C_{22}H_{22}N_6NiCl_2$ C, 52,84; H, 4.43; N, 16.81. Found (%): C, 52.69; H, 4.41; N, 16.73; IR (KBr, cm^{-1}): 1524 (C=N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.3 (m, $CH_2CH_2CH_2$-CN, 4H), 2.4 (t, $CH_2CH_2CH_2$-CN, 4H), 3.7 (t, $CH_2CH_2CH_2$-CN, 4H), 7.7-8.2 (m, Ar-H, 8H), 8.4 (s, CH, 2H).

Microbiology: Antibacterial and antifungal activities of the tested compounds were determined by using agar dilution procedure outlined by the National Committee for Clinical Laboratory standarts[23,24]. Minimal inhibitory concentrations (MIC) for each compound were investigated against standard bacterial strains; *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and yeast-like fungi; *Candida*

albicans and *Candida tropicalis* obtained from the Department of Microbiology Faculty of Medicine Ege University, Turkey. The stock solutions were prepared in dimethyl sulfoxide and DMSO had no effect on the microorganisms in the concentrations studied. All of the dilutions were done with distillated water. The concentrations of tested compounds were 800, 400, 200, 100, 50 and 25 μg/mL ampicillin and flucanazole from FAKO (Istanbul, Turkey) were used as a reference compound for the experimental conditions. A loopfull (0.01 mL) of the standardized inoculums of the bacteria and fungi (10^6 CFUs/mL) was spreaded over the surface of agar plates. All the inoculated plates were incubated at 35 °C and results were evaluated after 16-20 h for bacteria and 48 h for fungi. The lowest concentration of the compounds that prevented visible growth was considered to be minimal inhibitor concentrations (MICs).

RESULTS AND DISCUSSION

The structure of compounds synthesized were identified by ^{1}H NMR, FT-IR and micro analysis. ^{1}H NMR, FT-IR, yields, melting points and analytical data of the newly synthesized compounds are given in experimental section.

In the IR spectra of the complexes, noticeable changes are observed compare with the starting ligands, especially in the frequencies of the ν(C=N) vibrations. For example, upon complexation, ν(C=N) frequencies shift to the left as 15-45 cm^{-1} and the peaks weaken relatively. The stretching vibration frequencies of the aromatic ν(C=C) appear between 1488-1455 cm^{-1} as medium or sharp bands. Because of the paramagnetic properties of the cobalt(II) atom, we could not observed carbon peaks in the ^{13}C NMR spectra. ^{1}H NMR spectra were obtained but the peaks were board.

The antimicrobial and antifungal activity results (MIC value) of the complexes are given in Tables 1 and 2, respectively. Tables 1 and 2 also contain ampicillin and flucanozole reference compounds results, respectively to compare and the reliability of the method used.

In this study 13 new transition metal benzimidazole complexes were tested against standard strains of gram-positive and gram-negative bacteria (**Scheme-I**). As it can be seen in Table-1, the benzimidazole complexes **5**, **6**, **13**, **14**, **17** and **18** inhibited the growth of gram-positive bacteria with MICs between 100 and 400 μg/mL. None of the benzimidazole complexes show antimicrobial activity against gram negative bacteria studied in this work. As can be seen in Table-2, the compounds **3**, **5**, **6**, **13**, **14**, **15**, **16**, **17** and **18** also showed some activity against *C. albicanis* and *C. tropicalis* with a range of MICs between among 100 and 400 μg/mL. Among the tested compounds **5** and **6** were the most effective compound with MIC 100 μg/mL against *Candida albicanis* and *Candida tropicalis*.

Considering the structures of the tested complexes that exhibited antimicrobial activity, the substituted alkyl group attached N1-nitrogen may play an important role for the antibacterial and antifungal activity. Particularly, bearing cyano group

TABLE-1
MICs (μg/mL) OF THE TESTED COMPOUNDS

Compd. No.	Tested microorganisms			
	Enterococus faecalis (ATCC 29212)	*Staphylocucus aureus* (ATCC 29213)	*Escherichia coli* (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)
Ampicillin	0.78	0.39	3.12	>75
1	>800	800	>800	>800
2	>800	>800	>800	>800
3	>800	>800	>800	>800
4	800	800	>800	>800
5	200	400	>800	>800
6	400	800	>800	>800
7	800	800	>800	>800
8	>800	>800	>800	>800
9	>800	>800	>800	>800
10	800	800	>800	800
11	800	800	>800	>800
12	800	800	>800	800
13	400	400	>800	>800
14	400	400	>800	>800
15	800	800	>800	>800
16	800	800	>800	>800
17	100	200	>800	>800
18	200	200	>800	>800

TABLE-2
MICs (μg/mL) OF THE TESTED COMPOUNDS

Compd. No.	Tested organisms	
	Candida albicans	*Candida tropicalis*
Fluconazole	1.25	1.25
1	800	800
2	800	800
3	800	400
4	800	800
5	100	100
6	100	100
7	800	800
8	800	800
9	800	800
10	800	800
11	800	800
12	800	800
13	200	200
14	200	200
15	400	400
16	400	400
17	100	200
18	100	100

on alkyl chain such as compounds **5**, **17** and **18** were found to be more effective than others. From the data obtained in this work, it is also suggested that especially increasing the apolar character of the alkyl group may a crucial role in the antimicrobial activity against gram positive bacteria and yeast-like fungi.

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REFERENCES

1. H.S. Günes and G. Cosar, *Arzneim. Forsch/Drug Res.*, **42**, 1045 (1992).
2. J. Easmon, G. Puerstinger, T. Roth, H.H. Fiebig, M. Jenny, W. Jaeger, G. Heinisch and J. Hofmann, *Int. J. Cancer*, **94**, 89 (2001).
3. H. Küçükbay, R. Durmaz, E. Orhan and S. Günal, *IL Farmaco*, **58**, 431 (2003).
4. B. Çetinkaya, E. Çetinkaya, H. Küçükbay and R. Durmaz, *Arzneim. Forsch/Drug Res.*, **46**, 1154 (1996).
5. B. Çetinkaya, E. Çetinkaya, H. Küçükbay and R. Durmaz, *Arzneim. Forsch/Drug Res.*, **46**, 821 (1996).
6. H. Küçükbay and B. Durmaz, *Arzneim. Forsch/Drug Res.*, **47**, 667 (1997).
7. H. Küçükbay, E. Çetinkaya and R. Durmaz, *Arzneim. Forsch/Drug Res.*, **45**, 1331 (1995).
8. H. Küçükbay, R. Durmaz, N. Sireci and S. Günal, *Asian J. Chem.*, **22**, 2816 (2010).
9. H. Küçükbay, R. Durmaz, N. Okuyucu, S. Günal and C. Kazaz, *Arzneim. Forsch/Drug Res.*, **54**, 64 (2004).
10. H. Küçükbay, R. Durmaz, N. Okuyucu and S. Günal, *Folia Microbiol.*, **48**, 679 (2003).
11. R. Durmaz, H. Küçükbay, E. Çetinkaya and B. Çetinkaya, *Turk. J. Med. Sci.*, **27**, 59 (1997).
12. A.K. Tiwari, A.K. Mishra, A. Bajpai, P. Mishra, S. Singh, D. Sinha and V.K. Singh, *Bioorg. Med. Chem. Lett.*, **17**, 2749 (2007).
13. R.J. Sundberg and R.B. Martin, *Chem. Rev.*, **74**, 471 (1974).
14. B. Rosenberg and B. Lippert, Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug, Verlag Chemie VCH, Basel, Switzerland (1999).
15. I.H. Krakoff, Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy: Clinical Application of Platinum Complexes, M. Nicali, Martinus Nijhoff, Boston, USA (1988).
16. E. Lukevics, P. Arsenyan, I. Shestakova, I. Domracheva, A. Nesterova and O. Pudova, *Eur. J. Med. Chem.*, **36**, 507 (2001).
17. K.K. Monhilal, C. Karunakaran, A. Rajendran and R. Murugesan, *J. Inorg. Biochem.*, **98**, 322 (2004).
18. N.M. Agh-Atabay, B. Dulger and F. Gucin, *Eur. J. Med. Chem.*, **40**, 1096 (2005).
19. N.M. Agh-Atabay, A. Neshat, T. Karabiyik, M. Somer, D. Haciu and B. Dulger, *Eur. J. Med. Chem.*, **42**, 205 (2007).
20. B. Ülküseven, A. Tavman and G. Ötük, *Metal Based Drugs*, **6**, 163 (1999).
21. S. Türktekin, M. Akkurt, E. Orhan, F.Z. Küçükbay, H. Küçükbay and O. Büyükgüngör, *Acta Cryst.*, **E60**, m1220 (2004).
22. M. Akkurt, S. Karaca, H. Küçükbay, E. Orhan and O. Büyükgüngör, *Acta Cryst.*, **E61**, m41 (2005).
23. National Committee for Clinical Laboratory Standards (NCCLS), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard M7-A2, NCCLS, Villanova, PA, USA (1997).
24. NCCLS, Reference Method for Broth Diluation Antifungal Susceptibility Testing of Yeasts. Proposed Standard, Document M27-P, NCCLS, Villanova, PA, USA (1992).

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