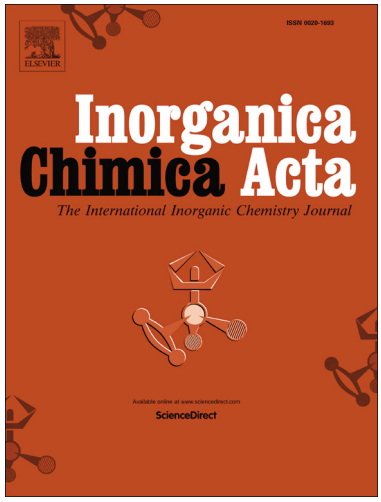

Research paper

Synthesis and evaluation of anticancer properties of novel benzimidazole ligand and their cobalt(II) and zinc(II) complexes against cancer cell lines A-2780 and DU-145

Ülkü Yılmaz, Suat Tekin, Nesrin Buğday, Kemal Yavuz, Hasan Kü ç ükbay, Süleyman Sandal

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Synthesis and evaluation of anticancer properties of novel benzimidazole ligand and their cobalt(II) and zinc(II) complexes against cancer cell lines A-2780 and DU-145

Ülkü Yılmaz[1,2], Suat Tekin[3], Nesrin Buğday[1], Kemal Yavuz[1], Hasan Küçükbay[1*], Süleyman Sandal[2]

[1]İnonu University, Art and Science Faculty, Department of Chemistry, Malatya, TURKEY

[2]Malatya Turgut Özal University, Battalgazi Vocational School, Battalgazi, Malatya, TURKEY

[2]Department of Physiology, Faculty of Medicine, İnönü University, Malatya, Turkey

Abstract

Eighteen new cobalt(II) or zinc(II) complexes of benzimidazole bearing 1-benzyl and 2-phenyl moieties were synthesized from the reaction of appropriate benzimidazole ligands and $CoCl_2$ or $ZnCl_2$. Their structural characterizations were done by IR, NMR (^1H, ^{13}C) and UV-VIS spectrometers. Cytotoxic activities of eighteen new complexes and three benzimidazole ligands were determined using A-2780 (human ovarian) and DU-145 (human prostate) cell lines. Antitumor properties of all compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell viability assay for the tested benzimidazole derivatives was performed and the $LogIC_{50}$ values of the compounds were calculated after a 24-hour treatment. All tested benzimidazole derivatives showed higher or comparable antitumor activity against A-2780 cell lines compared to the standard drug docetaxel with a $LogIC_{50}$ value of -0.81 μM ($p<0.05$). Eight of the examined compounds (**1**, **3**, **5**, **6**, **7**, **9**, **10** and **13**) showed high cytotoxic activity against A-2780 compared to the standard drug docetaxel. While the $LogIC_{50}$ of the docetaxel was -0.81 μM for A-2780 cells at 24 h, the IC_{50} values of compounds **1**, **3**, 5, **6**, **7**, **9**, **10** and **13** were -0.97, -1.30, -0.22, 0.13, -0.16, -0.73 and -0.53 μM,

respectively. Three of the compounds **1**, **18** and **V** showed high cytotoxic activity against DU-145 compared to docetaxel ($p<0.05$). While the $LogIC_{50}$ of the docetaxel was -1.13 µM for DU-145 cells at 24 h, the $LogIC_{50}$ values of compounds **1**, **18** and **V** were 0.84, -0.38 and -0.66 µM, respectively.

Keywords: Benzimidazole complexes, cytotoxic, A-2780 cell lines, DU-145 cell lines.

*Correspondence: hasan.kucukbay@inonu.edu.tr(HK)

1. Introduction

Today, drug resistance is one of the most important problems in the treatment of all diseases. Drug resistance also poses serious problems in cancer treatment. Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer. A correct cancer diagnosis is essential for adequate and effective treatment because every cancer type requires a specific treatment regimen that encompasses one or more modalities such as surgery, radiotherapy, and chemotherapy[1]. Therefore, there is a serious need for the synthesis of novel, effective, inexpensive, and low-side-effect drug candidates. Today, cisplatin (*cis*-diamminedichloroplatinum(II) is the most widely used anticancer drug in the treatment of various types of cancer despite the undesirable side effects and its high price. On the other hand, combination therapies with cisplatin and other metal-containing drugs are thought to overcome drug resistance and reduce toxicity [2]. Therefore, much attention has focused on designing new type of efficient and inexpensive anticancer agents with a broad range of anticancer activity with decreased side effects. In connection with these purposes, several benzimidazole derivatives and their metal complexes have been studied and obtained promising anticancer activity against several cell lines such as A549 (Lung) [3], A2780 (ovarian) [4], MCF-7 (breast) [5][6][7], BEAS-2B (bronchial epithelia) [5][3], HeLa (cervical-uterine) [6], PC3 (prostate) [4][5], DU-145 (prostate) [8],

HCT-15 (colon) [5], SMMC7721 (liver) [5], EC109 (esophagus) [9], U373 (glioblastoma) [5], SiHa (cervical) [10], SGC7901 (gastric) and HT1080 (fibrosarcoma) [11], HEK-293T (kidney) [12]. On the other hand, benzimidazoles, which are important biologically active compounds, have a wide variety of biological activities [13].

In our previous studies we have also synthesized and published many biologically active benzimidazole and benzimidazole metal complexes [14][15][16][17][18][19][3][20].

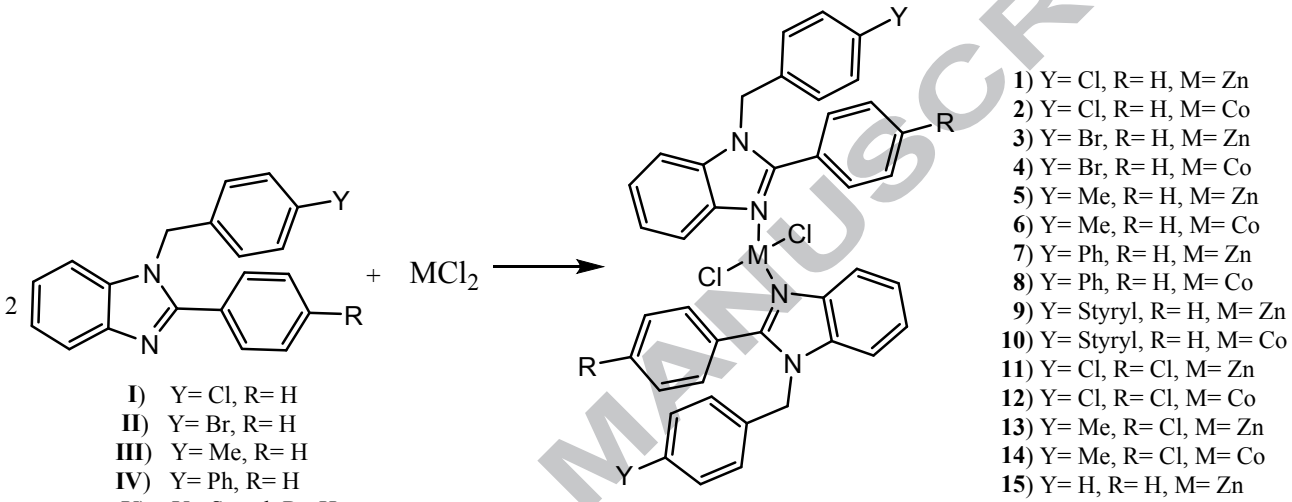
Metal complexes are generally more active than the corresponding ligands and there are some reports related with this situation in the literature [21][22].

According to the literature knowledge and our previous experiences on the benzimidazole derivatives, in this study it was aimed to synthesized new cobalt(II) and zinc(II) complexes incorporating 1-benzyl, 2-phenyl substituted benzimidazole moieties (Scheme 1) in an attempt to obtain new active compounds having cytotoxic activities against human cancer cell lines A-2780 and DU-145.

2. Experimental

The starting materials and reagents used in the reactions were supplied commercially by Aldrich or Merck Chemical Co. The solvents were dried by standard methods and freshly distilled prior to use. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a Bruker Avenced III 400 MHz Ultrashield high performance digital FT NMR spectrometer. Infrared spectra were recorded with ATR equipment in the range 4000 - 200 cm^{-1} on a Perkin-Elmer FT-IR spectrophotometer. Elemental analyses were performed by LECO CHNS-932 elemental analyzer. UV-Vis spectra were measured on a Perkin-Elmer Lambda 35 spectrophotometer. The melting points were recorded using an Electrothermal-9200 melting point apparatus and are uncorrected. Benzimidazole complexes **1-18** were synthesized from benzimidazole ligands (**I-IX**) with $CoCl_2.6H_2O$ or $ZnCl_2$ in EtOH. Benzimidazole ligands (**I**[1] [23], **II** [24], **III** [24], **IV**

[24], **VI** [25], **VII** [25], **VIII** [26], **IX** [27] were prepared according to the literature procedures. The benzimidazole ligand, (*E*)-2-phenyl-1-(4-styrylbenzyl)-1H-benzo[d]imidazole (**V**) was synthesized for the first time in this work using the Mizoroki-Heck reaction. similar to the literature method [28][29].



I) Y= Cl, R= H
II) Y= Br, R= H
III) Y= Me, R= H
IV) Y= Ph, R= H
V) Y= Styryl, R= H
VI) Y= Cl, R= Cl
VII) Y= Me, R= Cl
VIII) Y= H, R= H
IX) Y= H, R= Cl

1) Y= Cl, R= H, M= Zn
2) Y= Cl, R= H, M= Co
3) Y= Br, R= H, M= Zn
4) Y= Br, R= H, M= Co
5) Y= Me, R= H, M= Zn
6) Y= Me, R= H, M= Co
7) Y= Ph, R= H, M= Zn
8) Y= Ph, R= H, M= Co
9) Y= Styryl, R= H, M= Zn
10) Y= Styryl, R= H, M= Co
11) Y= Cl, R= Cl, M= Zn
12) Y= Cl, R= Cl, M= Co
13) Y= Me, R= Cl, M= Zn
14) Y= Me, R= Cl, M= Co
15) Y= H, R= H, M= Zn
16) Y= H, R= H, M= Co
17) Y= H, R= Cl, M= Zn
18) Y= H, R= Cl, M= Co

Scheme 1. Synthesis of novel benzimidazole metal complexes.

*1) Synthesis of (E)-2-phenyl-1-(4-styrylbenzyl)-1H-benzo[d]imidazole (**V**)*

A mixture of 1-(4-bromobenzyl)-2-phenylbenzimidazole (1.00 g, 2.75 mmol), styrene (0.31 g, 3.00 mmol), K_2CO_3 (0.83 g, 6.00 mmol), $Pd(OAc)_2$ (% 2 mmol) and PPh_3 (% 4 mmol) was heated in DMF (3 mL)-H_2O (3 mL) in a Schleck tube on a water bath for 6 h. The mixture was than cooled and the product extracted with ethyl acetate (25 mL X 3). The extracts were dried with anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was crystallized from ethanol to provide cream color crystals **V** 0.79 g (75%), mp: 171-172 °C. Anal Calcd for $C_{28}H_{22}N_2$ (386,5) (%): C 87.01, H 5.74, N 7.25. Found (%): C 86.90, H 5.65, N 7.32. $\nu_{(C=N)}$: 1614 cm^{-1}, $\nu_{(C=C)}$: 1511 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, 1H, Ar-H, *J*= 8 Hz), 7.64-7.62 (m, 2H, Ar-H), 7.43-7.38 (m, 6H, Ar-H, CH=CH), 7.29-7.25 (m, 3H, Ar-H), 7.18-7.16 (m, 4H, Ar-H), 7.02(d, 2H, Ar-H, *J*= 8 Hz), 7.01(s, 2H, Ar-H), 5.38 (s, -CH_2-, 2H) ppm. ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.7, 143.2, 137.4, 136.8, 136.7, 136.4, 130.6, 130.3,

129.5, 129.2, 129.1, 128.2(d), 127.3, 127.0, 126.9, 123.2, 122.7, 119.8, 111.6 (Ar-C and -CH=CH-), 47.8 (-CH_2-).

*2) Synthesis of dichlorobis(1-(4-chlorobenzyl)-2-phenyl-1H-benzimidazole-$_KN^3$)zinc(II) (**1**)*

A mixture of 1-(4-chlorobenzyl)-2-phenyl-1H-benzimidazole (**I**) (0.50 g, 1.57 mmol), $ZnCl_2$ (0.11 g, 0.80 mmol) and EtOH (20 mL) was heated under reflux for 5h. The mixture was filtered off while hot. The obtained cream color crude product was crystallized from EtOH/DMF (1:1). Yield: 0.49 g, 80%, mp: 242-243 °C. Anal Calcd for $C_{40}H_{30}Cl_4N_4Zn$ (773.9) (%): C 62.08, H 3.91, N 7.24. Found (%): C 61.82, H 3.84, N 7.15. $\nu_{(C=N)}$: 1599 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.79-7.77 (m, 2H, Ar-H), 7.73-7.70 (m, 4H, Ar-H), 7.55-7.52 (m, 6H, Ar-H), 7.50-7.48 (m, 2H, Ar-H), 7.36 (d, 4H, Ar-H, *J*= 8 Hz), 7.29-7.26 (m, 4H, Ar-H), 7.01 (d, 4H, Ar-H, *J*= 8 Hz), 5.59 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.9, 142.9, 136.3, 136.1, 132.6, 130.4, 130.2, 129.6, 129.3, 129.2, 128.5, 123.4, 122.9, 119.8, 111.6 (Ar-C), 47.3 (-CH_2-).

Similar to the procedure above, compounds **2**-**18** were synthesized from appropriate 1,2-disubstituted benzimidazole and $CoCl_2.6H_2O$ or $ZnCl_2$.

*3) Dichlorobis(1-(4-chlorobenzyl)-2-phenyl-1H-benzimidazole-$_KN^3$)cobalt(II) (**2**)*

Yield: 0.50 g, 83%, mp: 217-219 °C. Anal Calcd for $C_{40}H_{30}Cl_4N_4Co$ (767.4) (%): C 62.60, H 3.94, N 7.30. Found (%): C 62.40, H 3.90, N 7.21. $\nu_{(C=N)}$: 1603 cm^{-1}.

*4) Dichlorobis(1-(4-bromobenzyl)-2-phenyl-1H-benzimidazole-$_KN^3$)zinc(II) (**3**)*

Yield: 0.46 g, 78%, mp: 290-292 °C. Anal Calcd for $C_{40}H_{30}Br_2Cl_2N_4Zn$ (862.8) (%): C 55.68, H 3.50, N 6.49. Found (%): C 55.47, H 3.48, N 6.35. $\nu_{(C=N)}$: 1595 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.79-7.77 (m, 2H, Ar-H), 7.72-7.70 (m, 4H, Ar-H), 7.55-7.53 (m, 6H, Ar-H), 7.49 (d, 6H, Ar-H, *J*= 8 Hz), 7.29-7.26 (m, 4H, Ar-H), 6.95 (d, 4H, Ar-H, *J*= 8 Hz), 5.56 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.8, 142.9, 136.8, 136.1, 132.2, 130.4, 130.2, 129.6, 129.3, 128.8, 123.4, 122.9, 121.1, 119.8, 111.6 (Ar-C), 47.4 (-CH_2-).

*5) Dichlorobis(1-(4-bromobenzyl)-2-phenyl-1H-benzimidazole-$_KN^3$)cobalt(II) (**4**)*

Yield: 0.44 g, 75%, mp: > 300 °C. Anal Calcd for $C_{40}H_{30}Br_2Cl_2N_4Co$ (856.4) (%): C 56.10, H 3.53, N 6.54. Found (%): C 56.01, H 3.42, N 6.44. $\nu_{(C=N)}$: 1593 cm^{-1}.

*6) Dichlorobis(1-(4-methylbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)zinc(II) (**5**)*

Yield: 0.52 g, 85%, mp: 287-288 °C. Anal Calcd for $C_{42}H_{36}Cl_2N_4Zn$ (733.1) (%): C 68.82, H 4.95, N 7.64. Found (%): C 69.03, H 5.09, N 7.60. $\nu_{(C=N)}$: 1615 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.79-7.72 (m, 6H, Ar-H), 7.55-7.46 (m, 8H, Ar-H), 7.28-7.24 (m, 4H, Ar-H), 7.09 (d, 4H, Ar-H, *J*= 8 Hz), 6.89 (d, 4H, Ar-H, *J*= 8 Hz), 5.53 (s, 4H, -CH_2-), 2.23 (s, 6H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.9, 142.9, 137.2, 136.2, 134.2, 130.4, 129.8, 129.6, 129.3, 126.5, 123.3, 122.8, 119.7, 111.7 (Ar-C), 47.8 (-CH_2-), 21.1 (CH_3).

*7) Dichlorobis(1-(4-methylbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)cobalt(II) (**6**)*

Yield: 0.51 g, 84%, mp: 294-295 °C. Anal Calcd for $C_{42}H_{36}Cl_2N_4Co$ (726.6) (%): C 69.43, H 4.99, N 7.71. Found (%): C 69.39, H 5.08, N 7.93. $\nu_{(C=N)}$: 1615 cm^{-1}.

*8) Dichlorobis(1-(4-phenylbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)zinc(II) (**7**)*

Yield: 0.42 g, 70%, mp: 295-296 °C. Anal Calcd for $C_{52}H_{40}Cl_2N_4Zn$ (857.2) (%): C 72.86, H 4.70, N 6.54. Found (%): C 72.70, H 4.62, N 6.50. $\nu_{(C=N)}$: 1590 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.78-7.75 (m, 6H, Ar-H), 7.62-7.54 (m, 16H, Ar-H), 7.43 (t, 4H, Ar-H, *J*= 8 Hz), 7.35 (d, 2H, Ar-H, *J*= 8 Hz), 7.31-7.25 (m, 4H, Ar-H), 7.10 (d, 4H, Ar-H, *J*= 8 Hz), 5.64 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.8, 143.0, 139.9, 139.8, 136.5, 136.2, 130.5, 130.4, 129.6, 129.4, 129.3, 128.0, 127.6, 127.2, 127.0, 123.3, 122.9, 119.8, 111.7 (Ar-C), 47.7 (-CH_2-).

*9) Dichlorobis(1-(4-phenylbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)cobalt(II) (**8**)*

Yield: 0.43 g, 73%, mp: > 300 °C. Anal Calcd for $C_{52}H_{40}Cl_2N_4Co$ (850.8) (%): C 73.41, H 4.74, N 6.59. Found (%): C 73.20, H 4.60, N 6.48. $\nu_{(C=N)}$: 1609 cm^{-1}.

*10) Dichlorobis(1-(4-styryllbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)zinc(II) (**9**)*

Yield: 0.39 g, 66%, mp: 247-248 °C. Anal Calcd for $C_{56}H_{44}Cl_2N_4Zn$ (909.3) (%): C 73.97, H 4.88, N 6.16. Found (%): C 73.79, H 4.72, N 6.00. $\nu_{(C=N)}$: 1611 cm^{-1}, $\nu_{(C=C)}$: 1514 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.77-7.74 (m, 6H, Ar-H), 7.56-7.52 (m, 16H, Ar-H and HC=CH), 7.37 (t, 4H, Ar-H, *J*= 6 Hz), 7.28-7.26 (m, 6H, Ar-H), 7.20 (s, 4H, Ar-H), 7.01 (d, 4H, Ar-H), 5.60 (s, -CH_2-, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.8, 143.1, 137.4, 136.8, 136.7, 136.4, 130.6, 130.4, 129.6, 129.3, 129.2, 129.1, 128.2, 128.1, 127.3, 127.0, 126.9, 123.2, 122.8, 119.8, 111.6 (Ar-C and alkene-C), 47.8 (-CH_2-).

*11) Dichlorobis(1-(4-styryllbenzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)cobalt(II) (**10**)*

Yield: 0.40 g, 68%, mp: 260-261 °C. Anal Calcd for $C_{56}H_{44}Cl_2N_4Co$ (902.8) (%): C 74.50, H 4.91, N 6.21. Found (%): C 74.35, H 4.79, N 6.18. $\nu_{(C=N)}$: 1595 cm^{-1}, $\nu_{(C=C)}$: 1515 cm^{-1}.

*12) Dichlorobis(1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_K N^3$)zinc(II) (**11**)*

Yield: 0.47 g, 78%, mp: 276-277 °C. Anal Calcd for $C_{40}H_{28}Cl_6N_4Zn$ (842.8) (%): C 57.01, H 3.35, N 6.65. Found (%): C 56.88, H 3.20, N 6.45. $\nu_{(C=N)}$: 1651 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.78-7.72 (m, 6H, Ar-H), 7.61 (d, 4H, Ar-H, *J*= 8 Hz), 7.52-7.50 (m, 2H, Ar-H), 7.36 (d, 4H, Ar-H, *J*= 8 Hz), 7.29-7.27 (m, 4H, Ar-H), 7.02 (d, 4H, Ar-H, *J*= 8 Hz), 5.59 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 152.6, 142.9, 136.3, 135.3, 132.6, 131.3, 129.4, 129.3, 129.2, 128.5, 123.5, 123.0, 119.9, 111.6 (Ar-C), 47.3 (-CH_2-).

*13) Dichlorobis(1-(4-chlorobenzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_K N^3$)cobalt(II) (**12**)*

Yield: 0.48 g, 81%, mp: 290-291 °C. Anal Calcd for $C_{40}H_{28}Cl_6N_4Co$ (836.3) (%): C 57.45, H 3.37, N 6.70. Found (%): C 57.30, H 3.40, N 6.62. $\nu_{(C=N)}$: 1597 cm^{-1}.

*14) Dichlorobis(1-(4-methylbenzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_K N^3$)zinc(II) (**13**)*

Yield: 0.52 g, 87%, mp: 270-271 °C. Anal Calcd for $C_{42}H_{34}Cl_4N_4Zn$ (801.9) (%): C 62.91, H 4.27, N 6.99. Found (%): C 62.75, H 4.17, N 6.68. $\nu_{(C=N)}$: 1659 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.82-7.77 (m, 6H, Ar-H), 7.64 (d, 4H, Ar-H, *J*= 8 Hz), 7.54-7.51 (m, 2H, Ar-H), 7.33-7.28 (m, 4H, Ar-H), 7.12 (d, 4H, Ar-H, *J*= 8 Hz), 6.92 (d, 4H, Ar-H, *J*= 8 Hz), 5.57 (s, 4H, -CH_2-), 2.26 (s, 6H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 152.7, 142.8, 137.2, 136.3, 135.3, 134.1, 131.4, 129.8, 129.4, 129.3, 126.5, 123.5, 122.9,119.8, 111.8 (Ar-C), 47.8 (-CH_2), 21.1 (CH_3).

*15) Dichlorobis(1-(4-methylbenzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_K N^3$)cobalt(II) (**14**)*

Yield: 0.54 g, 90%, mp: 282-283 °C. Anal Calcd for $C_{42}H_{34}Cl_4N_4Co$ (795.5) (%): C 63.41, H 4.31, N 7.04. Found (%): C 63.22, H 4.23, N 7.03. $\nu_{(C=N)}$: 1602 cm^{-1}.

*16) Dichlorobis(1-benzyl)-2-phenyl-1H-benzimidazole-$_K N^3$)zinc(II) (**15**)*

Yield: 0.52 g, 84%, mp: 238-239 °C. Anal Calcd for $C_{40}H_{32}Cl_2N_4Zn$ (705.0) (%): C 68.15, H 4.58, N 7.95. Found (%): C 68.04, H 4.37, N 7.80. $\nu_{(C=N)}$: 1604 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.84 (d, 2H, Ar-H, *J*= 8 Hz), 7.74-7.71 (m, 4H, Ar-H), 7.53-7.48 (m, 8H, Ar-H),

7.29-7.23 (m, 10H, Ar-H), 7.00 (d, 4H, Ar-H, *J*= 8 Hz), 5.57 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 153.9, 137.2, 136.1, 130.5, 129.7, 129.3, 129.2, 128.0, 126.6, 123.4, 123.0, 119.7, 111.7 (Ar-C), 48.0 (-CH_2-).

17) Dichlorobis(1-benzyl)-2-phenyl-1H-benzimidazole-$_KN^3$)cobalt(II) ***(16)***

Yield: 0.50 g, 81%, mp: 278-279 °C. Anal Calcd for $C_{40}H_{32}Cl_2N_4Co$ (698.6) (%): C 68.78, H 4.62, N 8.02. Found (%): C 68.70, H 4.54, N 7.93. ν*(C=N)*: 1593 cm^{-1}.

18) Dichlorobis(1-benzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_KN^3$)zinc(II) ***(17)***

Yield: 0.47 g, 77%, mp: 238-239 °C. Anal Calcd for $C_{40}H_{30}Cl_4N_4Zn$ (773.9) (%): C 62.08, H 3.91, N 7.24. Found (%): C 62.22, H 3.93, N 7.03. $\nu_{(C=N)}$: 1601 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.81-7.79 (m, 2H, Ar-H), 7.75 (d, 4H, Ar-H, *J*= 8 Hz), 7.59 (d, 4H, Ar-H, *J*= 8 Hz), 7.52-7.50 (m, 2H, Ar-H), 7.31-7.23 (m, 10H, Ar-H), 6.99 (d, 4H, Ar-H, *J*= 4 Hz), 5.59 (s, 4H, -CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 152.7, 142.7, 137.2, 136.2, 135.3, 131.4, 129.4, 129.3, 129.1, 128.0, 126.6, 123.5, 123.0, 119.8, 111.7 (Ar-C), 48.0 (-CH_2-).

19) Dichlorobis(1-benzyl)-2-(4-chlorophenyl)-1H-benzimidazole-$_KN^3$)cobalt(II) ***(18)***

Yield: 0.47 g, 77%, mp: 238-239 °C. Anal Calcd for $C_{40}H_{30}Cl_4N_4Co$ (767.4) (%): C 62.60, H 3.94, N 7.30. Found (%): C 62.47, H 3.90, N 7.24. $\nu_{(C=N)}$: 1592 cm^{-1}.

4.5. Cell lines and culture conditions

The human cancer lines, ovarian cancer (A2780) and prostate cancer (DU-145) were used for in vitro screening experiments. The cell lines were both purchased from the American Type Culture Collection (ATCC). All cells were fed in 25 and 75 cm^2 flasks with RPMI-1640 medium (containing 10% fetal bovine serum, 100 U/ mL penicillin and 0.1 mg/mL streptomycin) in two days apart. In cells with a carbon dioxide (5% CO_2) incubator (Panasonic, Japan), the cells maintained at 37 °C and in a humid environment were separated from the flasks using a solution of trypsin-EDTA (Sigma-Aldrich, USA) when confluent. The viability of the

cells was determined using 0.4% trypan blue and experiments were started when the viability was above 90%.

4.5.1. MTT Assay

Antitumor activities of these substances were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[30]. Cells were removed using a trypsin-EDTA solution from flasks and counted by hemocytometer to determine cytotoxic effects. $15x10^3$ cells per well were plated in 96-well including 200 µL of RPMI-1640 medium. Cells were incubated at 37 °C in a CO_2 incubator for 24 hours to adhere to a 96-well plate base. When the incubation ended, concentrations of 0.1, 1, 10 and 100 10 µM of the benzimidazole derivatives were added to the wells in which the cells were contained. Incubation with cancer cells for 24 hours at 37 °C in a CO_2 incubator was performed to determine the effects of different concentrations of benzimidazole derivatives on cell viability for 24 hours. When the incubation was over, 0.5 mg/mL of MTT solution in sterile PBS was prepared and added to 96-well plates.

After MTT was added, the plates were incubated again for 3 hours. After this time, incubation was stopped by adding dimethylsulphoxide (DMSO) to the wells, and the optical densities of the cells in the plates were read on a spectrophotometer (Synergy HTX, USA) at a wavelength of 550 nm[31]. Cell viability percentage was calculated by proportioning the absorbance values obtained from benzimidazole ligands and their cobalt (II) and Zinc (II) complexes applied wells to that of control group. MTT trials were performed ten times in triplicate on different days and the inhibitory logarithmic 50 ($LogIC_{50}$) values of the applied compounds were calculated based on MTT results using the GraphPad prism 6 program on a computer.

Statical analysis

The IBM SPSS Statistics 24.0 (Windows) package program was used in the analysis. Conformity to normal distribution was evaluated by Shapiro Wilk test. Intergroup comparisons of quantitative variables were measured by Kruskal Wallis H test. When significant statistical differences were determined between groups, multiple comparisons were made with Bonferroni correction Mann Whitney U test. All *P* values <0.05 were considered statistically significant. $LogIC_{50}$ values of melatonin and agomelatine were calculated using Graphpad prism 6 program on the computer based on the MTT results obtained from the experiments.

3. Results and discussion

3.1. *Synthesis and characterization of the benzimidazole complexes*

The cobalt(II) and zinc(II) complexes of the benzimidazole derivatives were prepared from the reaction of the appropriate 1,2-disubstituted benzimidazole ligand and metal salt in good yields between 66-90%. The complexes were crystallized in a DMF/EtOH mixture. Benzimidazole ligand **V** was synthesized from the reaction of benzimidazole ligand **II** and styrene through the Heck-Mizoroki cross-coupling reaction with a good yield. The structures of Zn(II) benzimidazole complexes (**1**, **3**, **5**, **7**, **9**, **11**, **13**, **15** and **17)** were elucidated by ^{1}H NMR, ^{13}C NMR and IR spectrometric analyses. Due to paramagnetic properties of Co(II) benzimidazole complexes (**2**, **4**, **6**, **8**, **10**, **12**, **14**, **16** and **18**) we could not observe 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. While IR spectra of the compounds show the strong $\nu_{(C=N)}$ bands in free benzimidazoles at about 1600 cm^{-1} region, this bands shift slightly to higher wavelengths for the corresponding Co(II) and Zn(II) complexes. The red shift indicates the coordination of a tertiary nitrogen atom of the benzimidazole ligand to Co(II) and Zn(II) atoms. These types of red shifts are also reported in the literature [32] [33]. We have also observed these types of red

shift in our previous studies [34][35]. IR spectra of compound **V** showed the $\nu_{(C=N)}$ and $\nu_{(C=O)}$ stretching bands at 1614 and 1511 cm^{-1}, respectively, and these stretching bands were observed at 1611 and 1514 cm^{-1} for the corresponding Zn(II) complex and 1595 and 1515 cm^{-1} for the corresponding Co(II) complex. The $\nu_{(C=N)}$ and $\nu_{(C=O)}$ stretching frequencies of some other known benzimidazole ligands (**I**, **III** and **IX**) are reported as range of 1610 to 1635 cm^{-1} for $\nu_{(C=N)}$ and 1475 to 1495 cm^{-1} for $\nu_{(C=O)}$ in the literature [24].

While the characteristic CH_2 resonances for the benzyl group at position 1 of the benzimidazole ligands were observed between 5.37-5.49 ppm [24][25], they resonated between 5.53-5.64 ppm for the Zn(II) complexes of the benzimidazole ligands (**1**, **3**, **5**, **7**, **9**, **11**, **13**, **15** and **17)** . As expected, these benzylic methylene peaks shifted to downfield ($\Delta\delta\approx$ 0.15- to 0.16 ppm) for the Zn(II) complexes.

The general chemical shift to the downfield in both ^{1}H and ^{13}C NMR compared to the free benzimidazole ligands reveal the formation of the expected Zn(II) benzimidazole complexes.

The coordination to the Zn(II) ion for compound **9** shifts the ^{13}C NMR signals of the complex downfield from those of the free ligand **V** ($\Delta\delta\approx$ 0.1 ppm) for the carbon atom at position 2 of the benzimidazole ring. The carbon resonances for the carbon atom at position 2 of the benzimidazole ligands of Zn(II) complexes (**1**, **3**, **5**, **7**, **9**, **11**, **13**, **15** and **17)** were observed between 152.6-153.9 ppm. All other aliphatic and aromatic protons and carbons were observed in the expected regions. ^{1}H and ^{13}C NMR, FTIR and UV-VIS spectra of all compounds can be found in the supplementary information file provided with this manuscript.

The UV-Vis spectra of new benzimidazole complexes (**1**-**18**) and three benzimidazole ligands (**III**, **V** and **VII**) were determined in the 200-800 nm region in DMSO. Compound **III, V** and **VII** have absorption maxima at 323, 307, 267, 207; 332, 309, 297, 240 and 314, 310,

202, respectively, and these bands are attributed n- π* and π-π* transitions. In the complexes, these peaks are observed nearly similar. The d-d bands for all cobalt(II) complexes (**2**, **4**, **6**, **8**, **10**, **12**, **14**, **16** and **18**) were observed in the range of 670 (ϵ= 110 $M^{-1}.cm^{-1}$) to 679 nm (ϵ= 60 $M^{-1}.cm^{-1}$). All cobalt(II) complexes showed a single d-d 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*

Cytotoxic activities of eighteen new complexes and three benzimidazole ligands were determined using A-2780 (human ovarian) and DU-145 (human prostate) cell lines. In order to obtain the cytotoxic properties of the newly synthesized cobalt(II) and zinc(II) benzimidazole derivatives on A-2780 and DU-145 cells, the respective cell lines were incubated with increasing concentrations (0-100 μg/mL) of the compounds for 48 h and then subjected to an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. A time-dependent cell viability assay for the tested benzimidazole derivatives was performed and the $LogIC_{50}$ values of the compounds were calculated after a 24 h treatment. $LogIC_{50}$ values calculated for the A-2780 and DU-145 cells based on the results of MTT assays for a 24-hour interaction of benzimidazole derivatives are presented in Table 2.

Table 2 should be inserted here

As can be seen in Table 2, all tested benzimidazole derivatives showed higher or comparable antitumor activity against A-2780 cell lines compared to the standard drug docetaxel with a $LogIC_{50}$ value of -0.81 μM. Among the tested 18 complexes, those that incorporated zinc(II) metal and had chlorine, bromine or styryl at position 4 of the benzyl

substituent (**1**, **3**, **4** and **9**) showed higher cytotoxicity on A-2780 than the other compounds and the standard drug docetaxel at 0.1μM. A nearly similar result was obtained at 1 μM concentration for the A-2780 cell line. The cell viability results of A-2780 and DU-145 cells after a 48-h treatment with the three benzimidazole ligands (**III**, **V**, **VII**) and eighteen benzimidazole complexes of Zn(II) and Co(II) (**1**-**18**) are shown in Tables 3 and 4, respectively. As shown in Table 3, all tested benzimidazole derivatives demonstrated anticancer activity against the A-2780 cell line at 10 and 100 μM concentrations with $P < 0.05$, except compounds **12** and **16** at 10 μM concentration. Three of the compounds (**1**, **2** and **18)** showed antitumor activity against A-2780 cells at 0.1 μM concentration with $P< 0.05$. Thirteen of the tested compounds also exhibited antitumor activity against A-2780 cells at 10 μM concentration with $P< 0.05$. As can be seen in Tables 2 and 3, in accordance with literature information [21, 22], the metal complexes in this study generally showed better anticancer activity than the corresponding ligands on the A-2780 cell line. Three of the compounds **1**, **18** and **V** showed high cytotoxic activity against DU-145 compared to docetaxel as shown in Tables 2 and 4. While the $LogIC_{50}$ of the docetaxel was -1.13 μM for DU-145 cells at 24 h, the $LogIC_{50}$ values of compounds **1**, **18** and **V** were 0.84, -0.38 and -0.66 μM, respectively. In contrast to literature information [21, 22], anticancer activity against DU-145 cells was found more effective in ligand **V** than in the corresponding metal complexes **9** and **10**.

Tables 3 and 4 should be inserted here

4. Conclusions

Eighteen new benzimidazole Zn(II) and Co(II) metal complexes and three benzimidazole ligands (**V** is new) were synthesized successfully and their anticancer activities were evaluated on human cancer cell lines (A2780 and DU-145). Compounds **1**, **3**, **4** and **9** showed better

anticancer activity than the standard drug docetaxel at a concentration of 0.1 μM against the A2780 cell line. On the other hand, only **1**, **18** and **V** showed comparable anti-cancer activity at 100 μM concentration against DU145. In conclusion, the result of this study is encouraging us to continue our anticancer activity screening studies on benzimidazole derivatives.

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Declaration of interest

The authors declare no conflicts of interest.

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Table 1. Electronic absorption spectral bands and magnetic moments of novel benzimidazole ligands and their metal complexes.

Compounds	Electronic absorption bands* λ_{max} (nm)	
	Intraligand and charge transfer bands	d-d Bands
III	275, 250, 249	

V	278, 250, 248	
VII	312, 309, 282	
1	331, 284, 251	
2	282, 253, 242	671
3	275, 239, 233	
4	274, 243, 234	671
5	342, 278, 242	
6	279, 242, 233	679
7	279, 254, 233	
8	289, 248, 239	670
9	261, 243, 234	
10	364, 280, 246	678
11	282, 243, 230	
12	361, 280, 252	679
13	356, 280, 252	
14	386, 348, 333	678
15	279, 250, 231	
16	278, 254, 242	679
17	278, 246, 234	
18	356, 279, 247	678

* DMSO used as a solvent.

Table 2. LogIC_{50} (μM) concentrations calculated for A2780 and DU-145 cells in the GraphPad Prizm 6 program of benzimidazole derivatives.

LogIC$_{50}$ (µM) Concentration		
Comp. no	**A2780 cell**	**DU-145 cell**
1	-0.97	0.84
2	1.25	2.93
3	-1.30	1.83
4	1.13	4.42
5	-0.22	2.15
6	0.13	1.82
7	-0.16	1.06
8	1.39	12.60
9	-0.73	1.90
10	-0.53	2.03
11	1.52	5.59
12	2.28	2.97
13	0.41	6.44
14	1.60	2.92
15	1.24	2.32
16	2.94	1.66
17	2.18	1.97
18	3.18	-0.38
III	2.24	2.27
V	1.59	-0.66
VII	2.95	0.27
Docetaxel (Ref. Drug)	-0.81	-1.13

Table 3. The cell viability results of A2780 cells after a 48-h treatment with three benzimidazole

ligands (**III**, **V**, **VII**) and eighteen benzimidazole complexes of Zn(II) and Co(II) (**1**-**18**).†

A2780 Cell viability (%)						
Comp. no	**Control**	**Solvent (DMSO)**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
1	100±6	94±3	21±3*	25±2	21±4*	4±0*
2	100±6	94±3	76±7*	75±6*	72±8*	22±4*
3	100±6	94±3	43±7	19±6*	10±2*	4±0*
4	100±6	94±3	42±9	34±8*	30±4*	18±4*
5	100±6	94±3	98±15	27±3*	4±0*	4±0*
6	100±6	94±3	76±10	55±8*	27±4*	12±1*
7	100±6	94±3	67±7	57±6*	6±0*	5±0*
8	100±6	94±3	96±8	92±6	61±7*	30±4*
9	100±6	94±3	52±10	42±6*	10±1*	6±0*
10	100±6	94±3	60±5	38±6*	28±6*	22±9*
11	100±6	94±3	87±5	82±6*	50±5*	46±8*
12	100±6	94±3	97±6	95±3	95±3	64±11*
13	100±6	94±3	91±4	64±9*	28±5*	11±1*
14	100±6	94±3	90±5	82±3	58±6*	44±4*
15	100±6	94±3	95±4	90±7	73±6*	3±0*
16	100±6	94±3	94±2	89±8	89±4	67±5*
17	100±6	94±3	91±6	89±5	67±8*	55±6*
18	100±6	94±3	76±7*	73±11*	63±7*	59±4*
III	100±6	94±3	91±7	80±4*	70±3*	54±4*
V	100±6	94±3	92±6	66±6*	61±6*	44±6*
VII	100±6	94±3	96±3	80±7	68±6*	66±5*
Docetaxel (Ref. Drug)	100±6	94±3	54±6*	31±5*	10±1*	0±0*

* $P < 0.05$ 0.05

† The changes in cell viability caused by benzimidazole ligands and benzimidazole complexes are compared with the control data. Each data point is an average of 8 viability measurements.

Table 4. The cell viability results of DU-145 cells after a 48-h treatment with three

benzimidazole ligands (**III**, **V**, **VII**) and eighteen benzimidazole complexes of Zn(II) and Co(II) (**1**-**18**).‡

DU-145 Cell viability (%)						
Comp. no	**Control**	**Solvent (DMSO)**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
1	100±5	92±2	76±3*	63±10*	50±4*	29±4*
2	100±5	92±2	78±8*	66±7*	68±6*	55±9*
3	100±5	92±2	84±13	82±9*	78±8*	41±5*
4	100±5	92±2	84±17	77±7*	84±7	64±6*
5	100±5	92±2	95±2	94±4	69±5	28±6*
6	100±5	92±2	95±4	90±4	54±8*	52±6*
7	100±5	92±2	94±3	70±7	45±4*	36±4*
8	100±5	92±2	94±5	93±2	94±5	90±5
9	100±5	92±2	97±3	84±7	75±8*	46±5*
10	100±5	92±2	89±4	74±6*	67±7*	50±8*
11	100±5	92±2	84±5	79±8*	76±7*	71±8*
12	100±5	92±2	101±7	96±6	80±6	72±8*
13	100±5	92±2	104±4	104±4	102±4	102±5
14	100±5	92±2	97±5	94±4	97±3*	78±6
15	100±5	92±2	99±5	79±8	75±8*	56±8*
16	100±5	92±2	78±6*	78±8*	80±6*	33±5*
17	100±5	92±2	75±8*	75±6*	73±8*	42±7*
18	100±5	92±2	51±5*	53±6	39±10*	26±5*
III	100±5	92±2	87±3	87±4	92±5	55±6*
V	100±5	92±2	54±7*	40±5*	36±2*	24±2*
VII	100±5	92±2	80±5	46±11*	33±4*	31±4*
Docetaxel (Ref. Drug)	100±5	92±2	45±5*	28±4*	9±2*	0±0*

* $P < 0.05$ 0.05

‡ The changes in cell viability caused by benzimidazole ligands and benzimidazole complexes are compared with the control data. Each data point is an average of 8 viability measurements.

Highly active anticancer candidates against to A2780 cell lines were synthesized.

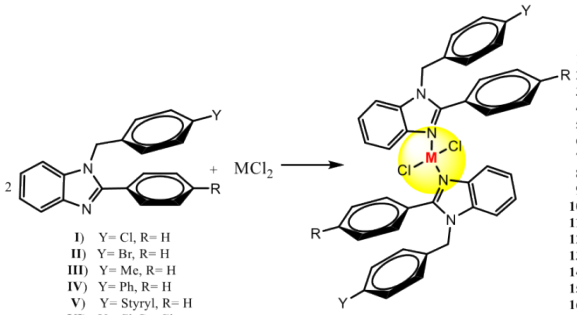
Anticancer properties against DU-145 cell lines of new complexes were tested.

New Zn (II) and Co (II) complexes of benzimidazole with anticancer activity.

Eighteen new cobalt (II) or zinc (II) complexes of benzimidazole bearing 1-benzyl and 2-phenyl moieties were synthesized from the reaction of appropriate benzimidazole ligands and $CoCl_2$ or $ZnCl_2$. Their structural characterizations were done by IR, NMR (^1H, ^{13}C) and UV-VIS spectrometers. Cytotoxic activities of eighteen new complexes and three benzimidazole ligands were determined using A-2780 (human ovarian) and DU-145 (human prostate) cell lines. Antitumor properties of all compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell viability assay for the tested benzimidazole derivatives was performed and the $LogIC_{50}$ values of the compounds were calculated after a 24-hour treatment. All tested benzimidazole derivatives showed higher or comparable antitumor activity against A-2780 cell lines compared to standard drug docetaxel with -0.81 μM $LogIC_{50}$ value ($p<0.05$). Eight of the examined compounds (**1**, **3**, **5**, **6**, **7**, **9**, **10** and **13**) showed high cytotoxic activity against A-2780 compared to standard drug docetaxel. While the $LogIC_{50}$ of the docetaxel was -0.81 μM for A-2780 cells at 24 h, the IC_{50} values of compounds **1**, **3**, 5, **6**, **7**, **9**, **10** and **13** were -0.97, -1.30, -0.22, 0.13, -0.16, -0.73 and -0.53 μM, respectively. Three of the compounds **1**, **18** and **V** showed high cytotoxic activity against DU-145 compared to docetaxel ($p<0.05$). While the $LogIC_{50}$ of the docetaxel was -1.13 μM for DU-145 cells at 24 h, the $LogIC_{50}$ values of compounds **1**, **18** and **V** were 0.84, -0.38 and -0.66 μM, respectively.

Synthesis and evaluation of anticancer properties of novel benzimidazole ligands and their cobalt (II) and Zinc (II) complexes against cancer cell lines A-2780 and DU-145.

Ülkü Yılmaz, Suat Tekin, Nesrin Buğday, Kemal Yavuz, Hasan Küçükbay*, Süleyman Sandal



I) Y= Cl, R= H
II) Y= Br, R= H
III) Y= Me, R= H
IV) Y= Ph, R= H
V) Y= Styryl, R= H
VI) Y= Cl, R= Cl
VII) Y= Me, R= Cl
VIII) Y= H, R= H
IX) Y= H, R= Cl

1) Y= Cl, R= H, M= Zn
2) Y= Cl, R= H, M= Co
3) Y= Br, R= H, M= Zn
4) Y= Br, R= H, M= Co
5) Y= Me, R= H, M= Zn
6) Y= Me, R= H, M= Co
7) Y= Ph, R= H, M= Zn
8) Y= Ph, R= H, M= Co
9) Y= Styryl, R= H, M= Zn
10) Y= Styryl, R= H, M= Co
11) Y= Cl, R= Cl, M= Zn
12) Y= Cl, R= Cl, M= Co
13) Y= Me, R= Cl, M= Zn
14) Y= Me, R= Cl, M= Co
15) Y= H, R= H, M= Zn
16) Y= H, R= H, M= Co
17) Y= H, R= Cl, M= Zn
18) Y= H, R= Cl, M= Co

Zn (II) and Co (II) complexes of benzimidazole with anticancer activity