Synthesis and Biological Activities of 2-Substituted Benzimidazole-Metal Complexes

Md. Afzal Azam, B. R. P. Kumar, R. Mazumdar and B. Suresh

Department of Pharmaceutical Chemistry, J. S. S., College of Pharmacy,
Ootacamund-643001, Tamil Nadu, India

ABSTRACT: A series of copper(II) and cobalt(II) coordination compounds with 2-substituted benzimidazole derived monodentate and bidentate ligands have been prepared and characterized by microanalysis, IR and UV-Vis spectroscopy. Synthesized metal complexes have been screened for their *in vitro* antioxidant and antitumor activity. The complex **4a** showed significant nitric oxide free radical scavenging activity (IC_{50} 65μg/ml), while **3i** and **3g** showed potent superoxide dismutase activity with IC_{50} of 0.26 and 0.28 μM respectively. *In vitro* cytotoxicity study with human breast MCF-7 and CNS SF 268 cancer cell lines showed that the most active 2-benzyl-1H-benzimidazole Cu(II) complex **3a** inhibited the growth of cancer cells at 20 μM concentration.

Keywords: Cu(II) complexes, Co(II) complexes, Benzimidazoles, Antitumor activity.

INTRODUCTION

2-Substituted benzimidazole derivatives are an important class of compounds in medicinal and organic chemistry. The benzimidazole moiety is a structural element of compounds with a wide range of biological activities[1-4]. For example, 2-substituted benzimidazoles exhibit inhibitory activity against a range of human tumour cell lines *in vitro*.[5-7] In addition transition metal complexes of 2-substituted benzimidazole ligands act as anticancer agents[8-12]. Cancer cells are found to have less superoxide dismutase activity than normal cells[13,14] and copper(II) complexes are known to mimic activity of copper, zinc-superoxide dismutase (Cu,Zn-SOD), an antioxidant enzyme that protect cells from the toxic effect of superoxide ion by its dismutation into dioxygen and hydrogen peroxide in biological systems[15]. The antitumor activity of SOD metal complexes has been suggested to be due to their superoxide scavenging ability[16]. Above observations prompted us to synthesize various 2-substituted benzimidazole-copper(II) and cobalt(II) complexes **3a-i** and **4a-i** as per Scheme 1 with a view to evaluate their anticancer and antioxidant activities.

Correspondence to: Md. Afzal Azam
E-mail: afzal9azam@hotmail.com
Fax: +91-423-2442937.

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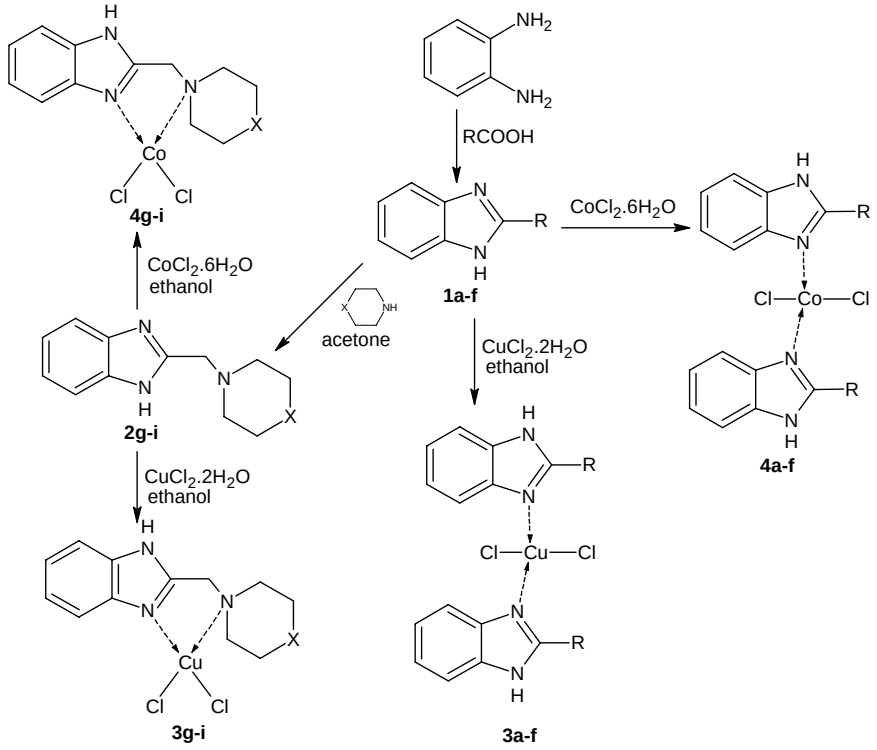
MATERIALS AND METHODS

Melting points were obtained in an open capillary tube. Structures of all the compounds were confirmed by elemental analysis and spectral data. The IR spectra were recorded on Shimadzu 800 FTIR spectrometer in KBr pellets. The ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 (90 MHz) spectrometer in $CDCl_3$ using TMS as an internal standard. All chemical shift values are expressed in δ ppm. Mass spectra were recorded on a Shimadzu 2010A LC-MS spectrometer and UV spectra were taken on Perkin Elmer version 2.5 spectrophotometer. Elemental analysis of compounds was carried out on Flash EA 1112 series instrument. The homogeneity of all the compounds were verified on silica gel G coated TLC plates and the spots were located by iodine vapours.



X = CH_2, $CH_2.(CH_3)$ and O

R= $CH_2.C_6H_5$, SH, $S.CH_2.C_6H_5$, $C_6H_5.NH_2$,CH_2 Cl and C_6H_5

Scheme 1: Synthesis of 2-substituted benzimidazole-metal complexes

Table 1. Characterization data of synthesized compounds 1a-f, 2g-i, 4a-i and 5a-i

Compound	(R)	X	Formula	MP (^{0}C)	*Yield (%)
1a	$CH_2C_6H_5$	---	$C_{14}H_{12}N_2$	191[17]	59
1b	CH_2Cl	---	$C_8H_7ClN_2$	147[22]	65
1c	$C_6H_4NH_2$	---	$C_{13}H_{11}N_3$	222	68
1d	C_6H_5	---	$C_{13}H_{10}N_2$	295[22]	71
1e	SH	---	$C_7H_6N_2S$	302[22]	73
1f	$S.CH_2C_6H_5$	---	$C_{14}H_{12}N_2S$	194	69
2g	---	CH_2	$C_{13}H_{17}N_3$	235	62
2h	--	$CH\text{-}CH_3$	$C_{14}H_{19}N_3$	263	70
2i	---	-O-	$C_{12}H_{15}N_3O$	233	64
4a	$CH_2C_6H_5$	---	$C_{28}H_{24}Cl_2CuN_4$	150	63
4b	CH_2Cl	---	$C_{16}H_{14}Cl_4CuN_4$	170	65
4c	$C_6H_4.NH_2$	---	$C_{26}H_{22}Cl_2CuN_6$	48	77
4d	C_6H_5	---	$C_{26}H_{20}Cl_2CuN_4$	230	71
4e	SH	---	$C_{14}H_{12}Cl_2CuN_4S_2$	72	65
4f	$S.CH_2C_6H_5$	---	$C_{28}H_{24}Cl_2CuN_4S_2$	120	67
4g	---	CH_2	$C_{13}H_{17}Cl_2CuN_3$	99	62
4h	---	$CH\text{-}CH_3$	$C_{14}H_{19}Cl_2CuN_3$	136	66
4i	---	-O-	$C_{12}H_{15}Cl_2CuN_3O$	123	64
3a	$CH_2C_6H_5$	---	$C_{28}H_{24}Cl_2CoN_4$	237	79
3b	CH_2Cl	---	$C_{16}H_{14}Cl_4CoN_4$	190	71
3c	$C_6H_4NH_2$	---	$C_{26}H_{22}Cl_2CoN_6$	70	71
3d	C_6H_5	---	$C_{26}H_{20}Cl_2CoN_4$	150	67
3e	SH	---	$C_{14}H_{12}Cl_2CoN_4S_2$	45	65
3f	$S.CH_2C_6H_5$	---	$C_{28}H_{24}Cl_2CoN_4S_2$	200	63
3g	---	CH_2	$C_{13}H_{17}Cl_2CoN_3$	136	62
3h	---	$CH\text{-}CH_3$	$C_{14}H_{19}Cl_2CoN_3$	198	61
3i	---	-O-	$C_{12}H_{15}Cl_2CoN_3O$	172	64

* Isolated yield. All compounds showed satisfactory elemental analysis. [17,22]Lit. M.P. in ^{0}C **1a**: 191; **1b**: 146-148; **1d**: 293-296; **1e**: 300-304.

Chemistry. Synthesis of 2-substituted-1H-benzimidazoles 1a-f was carried out according to the standard procedure[17] and characterization data is presented in Table 1.

1c: IR (KBr, cm $^{-1}$): 3420, 3360 (N-H), 3046 (Ar-H), 1635 (C=N), 1606 (C=C aromatic), 1265 (C-N). ^{1}H NMR (δ ppm) ($CDCl_3$): δ 7.8 (s, 1H, NH), 7.6 (s, 2H, NH), 6.9-7.1 (m, 8H, Ar-H). MS: m/z 209 (M^+). Calculated for $C_{13}H_{11}N_3$: 74.64 % C; 5.26 % H; 20.09 % N..Found: 74.68 % C; 5.23 % H; 20.05 % N; **1f**: IR (KBr, cm $^{-1}$): 3448 (N-H), 3072 (Ar-H), 2958 (C-H aliphatic), 1652 (C=N), 1584 (C=C aromatic), 1268 (C-N), 749 (monosubstituted benzene). ^{1}H NMR (δ ppm) ($CDCl_3$): δ 7.6 (s, 1H, NH) 6.8-7.3 (m, 9H, Ar-H), 4.2 (s, 2H, CH_2). MS: m/z 240 (M^+). Calculated for $C_{14}H_{12}N_2S$: 70.00 % C; 5.00 % H; 11.66 % N. Found: 70.03 % C; 4.98 % H; 11.68 % N.

Synthesis of 2-(alkyl/arylamine-1-ylmethyl)-1H-benzimidazoles ***2g-i*****:** To a mixture of 2-chloromethyl benzimidazole **1b** (0.01mol) and appropriate amine (0.01 mol) in 50 ml of dry acetone, anhydrous potassium carbonate (0.69 g, 0.005 mol) was added. The reaction mixture was stirred at room temperature for 10-12 h. The solid thus obtained was filtered, washed with water, dried and recrystallized from acetone: methanol (1:1); **2g**: IR (KBr, cm $^{-1}$): 3340 (N-H), 3060 (Ar-H), 2936, 2880 (C-H aliphatic), 1654 (C=N), 1618 (C=C aromatic), 1266 (C-N); ^{1}H NMR (δ ppm) ($CDCl_3$): δ 7.72 (s, 1H, NH), 6.85-7.63 (m, 4H, ArH), 3.65 (s, 2H, CH_2), 2.80 (m, 4H, 2xCH_2 piperidine), 1.92 (m, 6H, 3xCH_2 piperidine). MS: m/z 215 (M^+). Calculated for $C_{13}H_{17}N_3$: 72.55 % C; 7.90 % H; 19.53 % N. Found: 72.57 % C; 7.93 % H; 19.51 % N; **2h**: IR (KBr, cm $^{-1}$): 3442 (N-H), 3025 (Ar-H), 2921, 2862 (C-H aliphatic), 1665 (C=N), 1588 (C=C aromatic), 1253 (C-N); MS: m/z 229 (M^+). Calculated for $C_{14}H_{19}N_3$: 73.36 % C; 8.29 % H; 18.34 % N. Found: 73.38 % C; 8.31 % H; 18.35 % N; **2i**: IR (KBr, cm $^{-1}$): 3454 (N-H), 3042 (Ar-H), 2962, 2862 (C-H aliphatic), 1647 (C=N), 1595 (C=C aromatic), 1268 (C-N), 1116 (C-O-C); MS: m/z 217 (M^+). Calculated for $C_{12}H_{15}N_3O$: 66.35 % C; 6.91 % H; 19.35 % N. Found: 66.33 % C; 6.92 % H; 19.37 % N.

Synthesis of Dichlorobis[2-(alkyl/aryl/aralkyl/thioaralkyl-1ylmethyl)-1H-benzimidazoles] copper (II) 3a-f: Appropriate benzimidazole ligand **1a-f** (0.01 mol) was dissolved in ethanol (50 ml) and the resulting solution was treated with copper(II) chloride (0.005 mol) dissolved in dimethylformamide (2 ml). The reaction mixture was stirred at room temperature for 6 h. The solid that separated in each case was filtered, washed with ether, dried and recrystallized from dimethylformamide; **3a:** IR (KBr, cm $^{-1}$): 3325 (N-H), 3015 (Ar-H), 2890 (C-H aliphatic), 1632 (C=N), 1610 (C=C aromatic), 1272 (C-N), 740 (monosubstituted benzene); MS: m/z 551 (M^+). Calculated for $C_{28}H_{24}Cl_2CuN_4$: 60.98 % C; 4.35 % H; 10.16 % N. Found: 60.96 % C; 4.37 % H; 10.14 % N; **3b**: IR (KBr, cm $^{-1}$): 3210 (N-H), 3015 (Ar-H), 2926 (C-H aliphatic), 1627 (C=N), 1605 (C=C aromatic), 1255 (C-N), 744 (C-Cl); MS: m/z 468 (M^+). Calculated for $C_{16}H_{14}Cl_4CuN_4$: 41.02 % C; 2.99 % H; 11.96 % N. Found: 41.02 % C; 2.97 % H; 11.94 % N; **3c**: IR (KBr, cm $^{-1}$): 3258, 3190 (N-H), 3012 (Ar-H), 1622 (C=N), 1594 (C=C aromatic), 1279 (C-N); MS: m/z 553 (M^+). Calculated for $C_{26}H_{22}Cl_2CuN_6$: 56.42 % C; 3.97 % H; 15.18 % N. Found: 56.40 % C; 3.95 % H; 15.15 % N; **3d**: IR (KBr, cm $^{-1}$): 3218 (N-H), 3082 (Ar-H), 1615 (C=N), 1595 (C=C aromatic), 1274 (C-N), 748 (monosubstituted benzene); MS: m/z 523 (M^+). Calculated for $C_{26}H_{20}Cl_2CuN_4$: 39.65 % C; 3.82 % H; 10.70 % N. Found: 39.64 % C; 3.85 % H; 10.68 % N; **3e**: IR (KBr, cm $^{-1}$): 3348 (N-H), 3030 (Ar-H), 2565 (SH), 1622 (C=N), 1605 (C=C aromatic), 1240 (C-N); MS: m/z 435 (M^+). Calculated for $C_{14}H_{12}Cl_2CuN_4S_2$: 38.62 % C; 2.75 % H; 12.87 % N. Found: 38.60 % C; 2.73 % H; 12.89 % N; **3f**: IR (KBr, cm $^{-1}$): 3285 (N-H), 3020 (Ar-H), 2928 (C-H aliphatic), 1635 (C=N), 1606 (C=C aromatic), 1286 (C-N), 740 (monosubstituted benzene); MS: m/z 615 (M^+). Calculated for $C_{28}H_{24}Cl_2CuN_4S_2$: 54.63 % C; 3.90 % H; 9.10 % N. Found: 54.61 % C; 3.93 % H; 9.12 % N.

The same procedure was applied for the preparation of other complexes ***4a-f*** **4a***:* IR (KBr, cm $^{-1}$): 3225 (N-H), 3040 (Ar-H), 2865 (C-H aliphatic), 1621 (C=N), 1603 (C=C aromatic), 1274 (C-N), 746 (monosubstituted benzene); MS: m/z 546 (M^+). Calculated for $C_{28}H_{24}Cl_2CoN_4$: 61.53 % C; 4.39 % H; 10.25 % N. Found: 61.50 % C; 4.41 % H; 10.26 % N; **4b**: IR (KBr, cm $^{-1}$): 3260 (N-H), 3060 (Ar-H), 2980 (C-H aliphatic), 1624 (C=N), 1604 (C=C aromatic), 1289 (C-N). MS: m/z 464 (M^+). Calculated for $C_{16}H_{14}Cl_4CoN_4$: 41.46 % C; 3.02 % H; 12.09 % N. Found: 41.49 % C; 3.03 % H; 12.04 % N; **4c**: IR (KBr, cm $^{-1}$): 3255, 3198 (N-H), 3014 (Ar-H), 1624 (C=N), 1610 (C=C aromatic), 1281 (C-N); MS: m/z 548 (M^+). Calculated for $C_{26}H_{22}Cl_2CoN_6$: 56.93 % C; 4.01 % H; 15.32 % N. Found: 56.95 % C; 4.05 % H; 15.30 % N; **4d**: IR (KBr, cm $^{-1}$): 3275 (N-H), 3068 (Ar-H), 1618 (C=N), 1607 (C=C aromatic), 1272 (C-N), 749 (monosubstituted benzene); MS: m/z 518 (M^+). Calculated for $C_{26}H_{20}Cl_2CoN_4$: 60.23 % C; 3.86 % H; 10.81 % N. Found: 60.26 % C; 3.87 % H; 10.79 % N; **4e**: IR (KBr, cm $^{-1}$): 3324 (N-H), 3059 (Ar-H), 2560 (SH), 1626 (C=N), 1590 (C=C aromatic), 1265 (C-N); MS: m/z 430 (M^+). Calculated for $C_{14}H_{12}Cl_2CoN_4S_2$: 39.06 % C; 2.79 % H; 13.02 % N. Found: 39.09 % C; 13.00 % H; 3.03 % N; **4f**: IR (KBr, cm $^{-1}$): 3197 (N-H), 3057 (Ar-H), 2964 (C-H aliphatic), 1635 (C=N), 1617, 1601 (C=C aromatic), 1269 (C-N); MS: m/z 610 (M^+). Calculated for $C_{28}H_{24}Cl_2CoN_4S_2$: 55.08 % C; 3.93 % H; 9.18 % N. Found: 55.06 % C; 3.96 % H; 9.17 % N.

Synthesis of *Dichloro[2-(alkyl/arylamine-1-ylmethyl)-1H-benzimidazoles] copper(II)* ***3g-i*****:** Appropriate benzimidazole ligand **2g-i** (0.005 mol) was dissolved in ethanol (50 ml) and the resulting solution was treated with copper(II) chloride (0.005 mol) dissolved in dimethylformamide (2 ml). The reaction mixture was stirred for 10 h at room temperature. The solid that separated in each was filtered, washed with ether, dried and recrystallized from dimethylformamide; **3g**: IR (KBr, cm $^{-1}$): 3221 (N-H), 3025 (Ar-H), 2968, 2854 (C-H aliphatic), 1622 (C=N), 1598 (C=C aromatic), 1275 (C-N); MS: m/z 350 (M^+). Calculated for $C_{13}H_{17}Cl_2CuN_3$: 44.57 % C; 4.86 % H; 12.00 % N. Found: 44.55 % C; 4.88 % H; 12.05 % N; **3h**: IR (KBr, cm $^{-1}$): 3280 (N-H), 3030 (Ar-H), 2980, 2860 (C-H aliphatic), 1642 (C=N), 1615 (C=C aromatic), 1265 (C-N); MS: m/z 364 (M^+). Calculated for $C_{14}H_{19}Cl_2CuN_3$: 46.15 % C; 5.21 % H; 11.54 % N. Found: 46.19 % C; 5.19 % H; 11.51 % N; **3i**: IR (KBr, cm $^{-1}$): 3202 (N-H), 3056 (Ar-H), 2921, 2850 (C-H aliphatic), 1629 (C=N), 1604 (C=C aromatic), 1271 (C-N), 1112 (C-O-C); MS: m/z 352 (M^+). Calculated for $C_{12}H_{15}Cl_2CuN_3O$: 40.90 % C; 4.26 % H; 11.93 % N. Found: 40.95 % C; 4.24 % H; 11.90 % N.

The same procedure was applied for the preparation of other complexes **4g-i. 4g**: IR (KBr, cm $^{-1}$): 3140 (N-H), 3015 (Ar-H), 2916 (C-H aliphatic), 1622 (C=N), 1596 (C=C aromatic), 1250 (C-N); MS: m/z 345 (M^+). Calculated for $C_{13}H_{17}Cl_2CoN_3$: 45.21 % C; 4.92 % H; 12.17 % N. Found: 45.20 % C; 4.94 % H; 12.15 % N; **4h**: IR (KBr, cm $^{-1}$): 3244 (N-H), 3032 (Ar-H), 2958, 2865 (C-H aliphatic), 1643 (C=N), 1608 (C=C aromatic), 1268 (C-N); MS: m/z 359 (M^+). Calculated for $C_{14}H_{19}Cl_2CoN_3$: 46.79 % C; 5.29 % H; 11.69 % N. Found: 46.76 % C; 5.30 % H; 11.67 % N; **4i**: IR (KBr, cm $^{-1}$): 3244 (N-H), 3028 (Ar-H), 2924 (C-H aliphatic), 1622 (C=N), 1601 (C=C aromatic), 1232 (C-N), 1118 (C-O-C). MS: m/z 347 (M^+). Calculated for $C_{12}H_{15}Cl_2CoN_3O$: 41.49 % C; 4.32 % H; 12.10 % N. Found: 41.46 % C; 4.30 % H; 12.08 N.

UV-Vis absorption study. UV-Vis absorption study of complexes **3a** and **4a** at 4 μM concentration was carried out with respect to their corresponding ligands **1a** and **4a**, respectively in phosphate buffer saline solution (Dulbecco's buffer, pH 7.4), pre-warmed at 37^{0}C (Figure 1 and 2). 10 μl of 20 mM DMF solution of the copper (**3a**) and cobalt (**4a**) complexes was added separately to Dulbecco's buffer (pH 7.4), pre-warmed at 37^{0}C, resulting in a final concentration of 4 μM. Spectra at 0, 1, 2 and 3 h were recorded between 240 and 400 nm. The resulting stability profile is shown in figure 3 and 4.

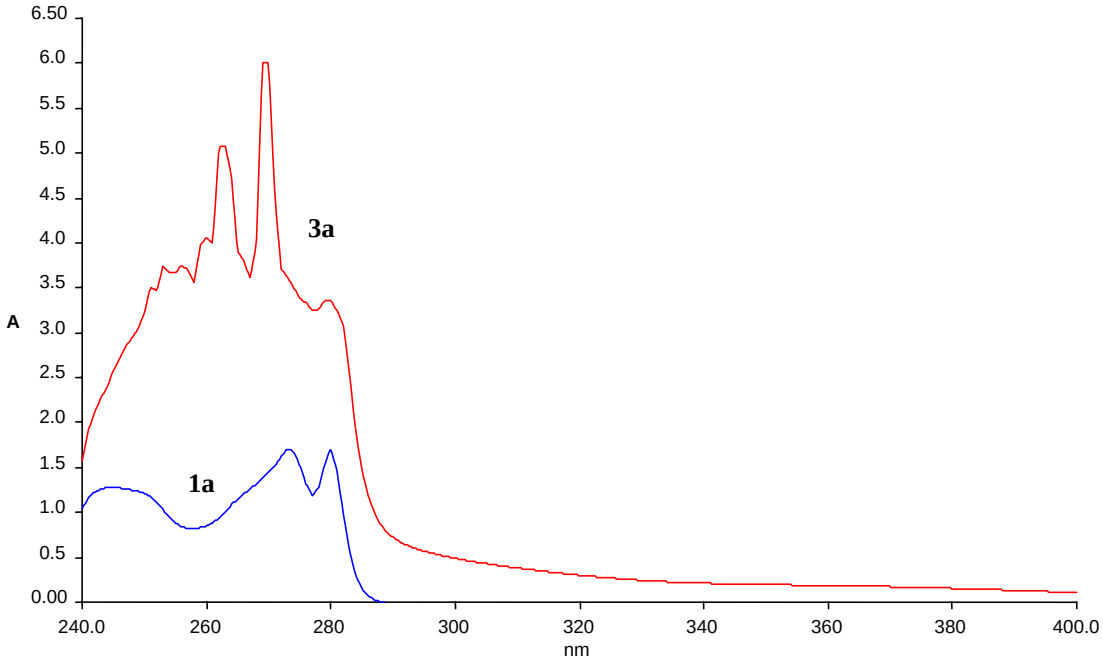


Figure 1. UV profile of compounds **1a** and **3a** in Dulbecco's buffer (pH 7.4, 37^{0}C)

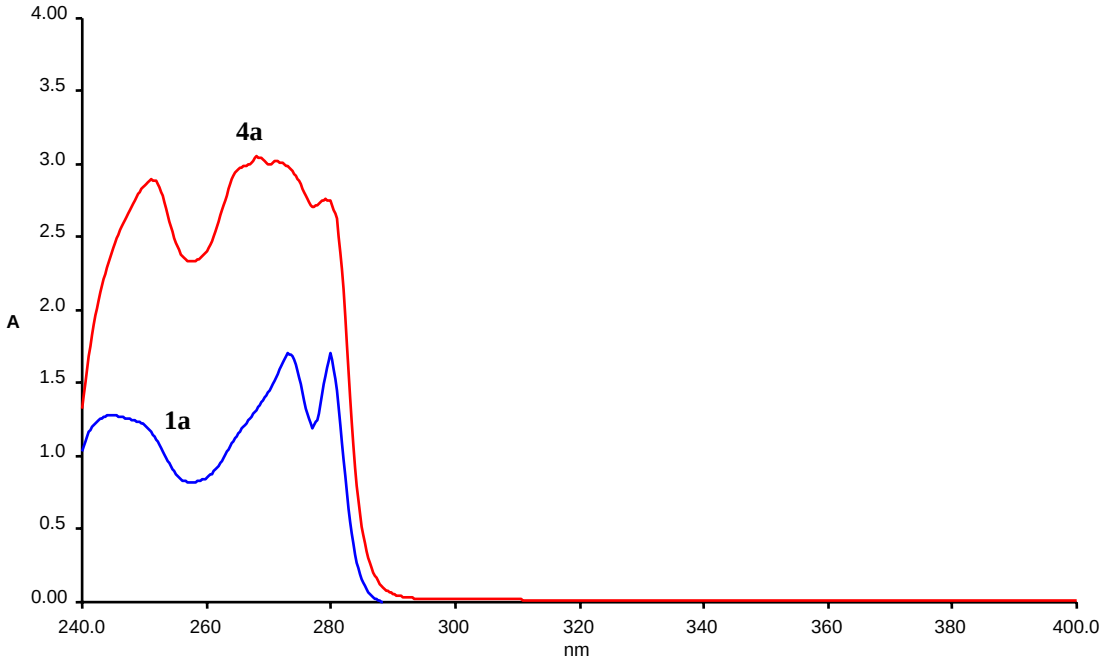


Figure 2. UV profile of compounds **1a** and **4a** in Dulbecco's buffer (pH 7.4, 37 ^{0}C)

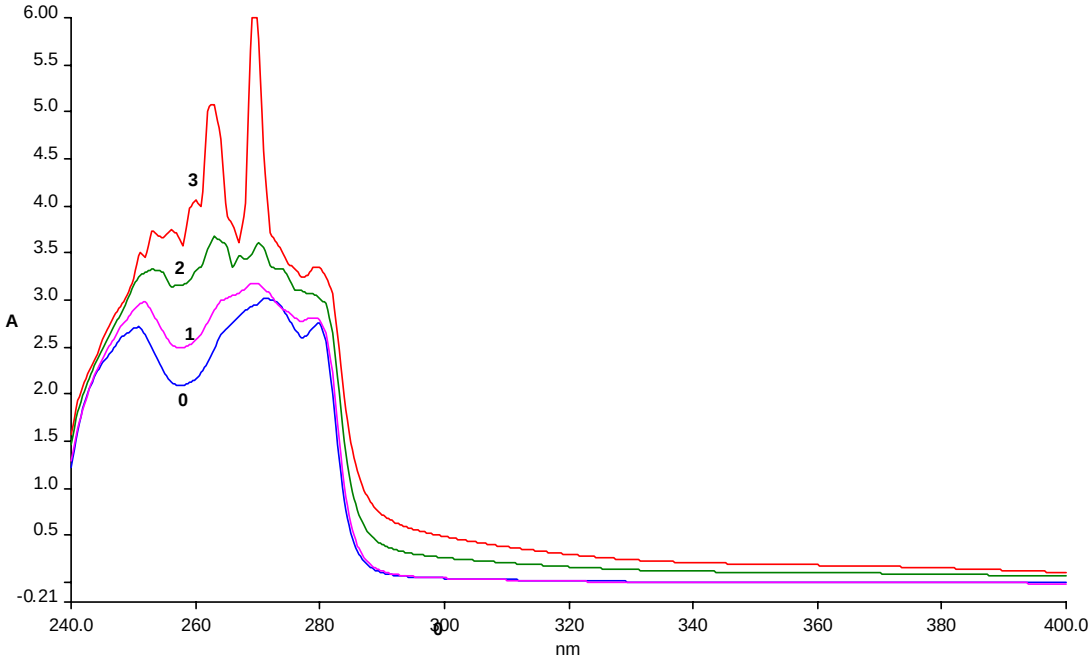


Figure 3. Stability profile of compound **3a** in Dulbecco's buffer (pH 7.4, 37 ^{0}C) at 0, 1, 2 and 3 h

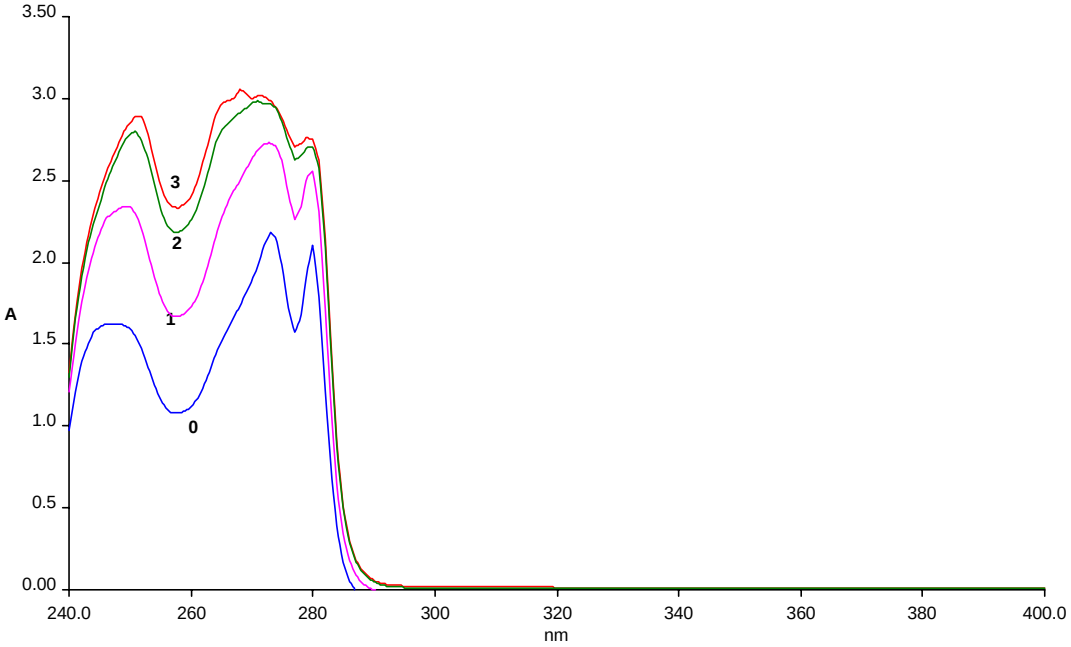


Figure 4. Stability profile of compound **4a** Dulbecco's buffer (pH 7.4, 37 ^{0}C) at 0, 1, 2 and 3 h

Biological evaluation. DPPH radical scavenging assay: Compounds 3a-i and 4a-i were evaluated as DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavengers as described by Hwang *et. al.*[18] 10 µl of the compound solution in a 1:1 DMSO/phosphate buffer (PH 7.4) mixture and 0.2 ml of DPPH in methanol solution was incubated at 37 ^{0}C for 30 min and the absorbance of the supernatant was measured at 490 nm. IC_{50} values were determined for the complexes 3a-i and 4a-i and the result is summarized with reference standard ascorbic acid in Table 2.

Nitric oxide radical inhibition assay: Nitric oxide radical inhibition assay of compounds **3a-i** and **4a-i** was assayed[19] by incubating 2 ml sodium nitroprusside (10 mM), 0.5 phosphate buffer saline and 0.5 ml (0.25 mg) of compound solution in a 1:1 DMSO/phosphate buffer (PH 7.4) mixture at 25 ^{0}C for 150 min. Then 1 ml of sulfanilic acid reagent was added to 0.5 ml of reaction mixture for 5 min to complete diazotization. Subsequently, 1 ml naphthyl ethylene diamine dihydrochloride (NEDD) was added and allowed to stand for 30 min at 25 ^{0}C. The absorbance of these solutions was measured at 540 nm. IC_{50} values were determined for the complexes **3a-i** and **4a-i** and the result is summarized with reference standard ascorbic acid in Table 2.

Superoxide dismutase (SOD) activity: Super oxide dismutase (SOD) activity of complexes **3a-i** and **4a-i** was determined in a 96 well microtitre plate assay based on the ability of the complexes to inhibit the reduction of nitroblue tetrazolium salt (NBT) by superoxide ions, which are generated by the traditional xanthine/xanthine oxidase system[20]. Superoxide radical anions reduce NBT to a blue formazan, which was detected spectrophotometrically at 570 nm. Solutions of the synthesized compounds at four different concentrations were prepared in a 1:1 DMSO/phosphate buffer (PH 7.4) mixture and 10 µl were added to 8 wells per concentration to give a concentration range between 0.01 and 1µM. Atleast three independent assays were performed for each complex and IC_{50} values were calculated for the complexes **3a-i** and **4a-i** and the result is summarized with reference standard rutin in Table 2.

Table 2. Anticancer and antioxidant activity of compounds 4a-i and 5a-i

Compound	*Antioxidant activity			** The average percent cell growth values	
	DPPH IC_{50} (µg/ml)	Nitric oxide IC_{50} (µg/ml)	SOD activity IC_{50} (µM)	MCF 7 cell line	SF 268 cell line
3a	51	>500	0.65	0.00	15.43
3b	>500	211	0.98	79.98	85.35
3c	>500	250	0.62	34.44	68.44
3d	>500	200	0.90	94.44	83.67
3e	>500	>500	0.59	77.50	78.23
3f	>500	>500	0.47	43.55	78.55
3g	>500	196	0.34	97.30	96.45
3h	>500	>500	0.44	97.40	98.55
3i	>500	76	0.26	97.14	80.25
4a	>500	65	0.32	56.54	75.65
4b	>500	165	0.56	44.54	56.25
4c	>500	>500	0.43	12.43	60.68
4d	>500	>500	0.52	24.65	55.68
4e	>500	>500	0.49	98.55	92.35
4f	>500	>500	0.48	55.23	56.25
4g	>500	>500	0.28	12.33	15.35
4h	>500	>500	0.58	58.23	60.55
4i	>500	>500	0.65	66.21	70.35
Standard drug	14 (Ascorbic acid)	69 (Rutin)	0.45 (Rutin)	---	---

* Average of three determinations. **Average of three determinations at 20 µM against human breast MCF 7 and CNS SF 268 cancer cell lines, zero means all cells are dead, the untreated controls showed 100 % growth.

In vitro antitumor activity: Antitumor activity of the newly synthesized compounds were evaluated by tryphan blue dye exclusion technique[21] against human breast MCF-7 and CNS SF 268 cancer cell lines at the concentration level of 20 μM. Each cell line is preincubated on microtitre plate, the test compounds are then added at 20 μM concentration. Cultures are incubated for fourty eight hours. Results for each compound are reported as the percent growth of the treated cells when compared to the untreated control cells. Primary screening of the compounds was done to indicate whether a compound possessed enough activity at this concentration to inhibit cell growth by 50 %. Results are given in Table 2.

RESULTS AND DISCUSSION

The required starting material 2-substituted-1H-benzimidazoles **1a-f** was prepared[17] by refluxing a mixture of *o*-phenylenediamine dihydrochloride and suitable aliphatic/aromatic carboxylic acid. Compounds **1a-f** when stirred with copper(II) and cobalt(II) chlorides separately in aqueous ethanol resulted in the formation of dichlorobis[2-substituted benzimidalzoles] copper(II) **3a-f** and dichlorobis [2-substituted benzimidazoles] cobalt(II) **4a-f**, respectively. 2-Chloromethyl-1H-benzimidazole **1b** was condensed with appropriate amine in dry acetone to furnish 2-(amino-1-ylmethyl)-1H-benzimidazoles **2g-i**. Further treatment of **2g-i** with copper(II) chloride and cobalt(II) chloride yielded dichloro[2-(amino-4ylmethyl)-1H-benzimidazoles] copper(II) **3g-i** and dichloro[(2-(amino-4ylmethyl)-1H-benzimidazoles] cobalt(II) **4g-i**, respectively. All the ligands synthesized were characterized by their IR, mass, ^{1}H NMR spectroscopy and elemental analysis. Metal complexes synthesized were characterized by IR, UV and elemental analysis. Elemental analysis suggested 1:2 (metal:ligand) stoichiometry for **3a-i** and **4a-i** and 1:1 (metal : ligand) stoichiometry for **3g-i** and **4g-i** complexes. IR spectrum of the complexes has shown some characteristic changes when compared to the free ligands. Ligands showed broad bands in the region 3460-3340 cm^{-1} due to the intermolecular hydrogen bonded imidazole N-H stretching. All the complexes which have free imidazole N-H, exhibited N-H stretching bands ranging from 3322 to 3140 cm^{-1} sharper than those of the ligands due to breaking of tautomerism, indicating that imidazole N-H was not involved in the coordination. Benzimidazole ligands exhibit strong to medium intensity bands in the region 1665-1630 cm^{-1} (C=N stretching), undergo a negative shift of 10-25 cm^{-1} which indicates involvement of azomethine nitrogen upon complexation. The other bands in the spectrum of each complex were similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

Figure 1 and 2 presents representative UV-Vis spectra of two pairs of ligands and complexes. The absorptions in the UV region are comparable for both the ligands and complexes of copper(II) and cobalt(II). The absorptions of the copper(II) and cobalt(II) complexes, however, extend further out into the visible region >380 nm. Fig. 3 and 4 show the time-dependant changes in the UV-Vis spectra of the two of the complexes over 3 h when they were incubated at 37 ^{0}C, in phosphate buffered saline. Compounds **3a** and **4a** showed a slow decrease in the intensity of the shape of the spectra over 3 h incubation but no change in the shape of the spectra and no crossing of the spectra was observed.

It is evident from result (Table 2) that only Copper(II) complex **3a** exhibited moderate (IC_{50} 51 μg/ml) DPPH free radical scavenging activity while all other compounds were found to be inactive (IC_{50} >500 μg/ml) in comparison to the reference standard ascorbic acid (IC_{50} 41 μg/ml). In nitric oxide method compounds **3i** and **4a** showed significant free radical scavenging activity with an IC_{50} of 76 μg/ml and 65 μg/ml, respectively in comparison to the reference standard rutin (IC_{50} 69 μg/ml). Compounds **3b**, **3c**, **3d**, **3g** and **4b** exhibited moderate activity while all other compounds were found to be inactive and no correlation was observed between nitric oxide free

radical scavenging activity, different substituents and Cu(II) and Co(II) complexes.

It is evident from superoxide dismutase activity result (Table 2) that compounds **3f-i**, **4a**, **4c** and **4e-g** exhibited potent activity (IC_{50} 0.26-0.49 μM) while all other compounds were found to be moderately active in comparison to the reference standard rutin (IC_{50} 0.45 μM). Copper(II) complex **3i** bearing morpholinomethyl group (IC_{50} 0.26 μM) and co(II) complex **4g** bearing piperidinomethyl group (IC_{50} 0.28 μM) showed maximum superoxide dismutase activity.

As shown in Table 2 metal complexes **3a**, **3c**, **3f**, **4b-d** and **4g** exhibited significant activity against human breast cancer MCF-7 cell line. Copper(II) complex bearing benzyl (**3a**) and cobalt(II) complexes bearing *p*-aminophenyl (**4c**) or piperidinomethyl (**4g**) substituents at position 2 of benzimidazole ring showed potent cytotoxicity against both human breast MCF 7 and human CNS SF 268 cancer cell lines while complexes **3c**, **4b** and **4d** exhibited moderate cytotoxicity against human breast cancer cell lineMCF-7. As evident from result no correlation was observed between corresponding superoxide dismutase (SOD) IC_{50} values and cytotoxic activity. This indicates that mechanisms other than SOD mimicking activity are responsible for their cytotoxic properties. Copper(II) complex **3i** showed significant SOD mimicking activity but no cytotoxicity on MCF-7 and SF 268 cancer cell lines at 20 μM might be useful therapeutically as a SOD mimicking agent.

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