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Anticancer activities of manganese-based photoactivatable CO-releasing complexes (PhotoCORMs) with benzimidazole derivative ligands

Elvan Üstün[1] · Aykut Özgür[2] · Kübra A. Coşkun[2] · Serpil Demir Düşünceli[3] · İsmail Özdemir[3] · Yusuf Tutar[4]

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Abstract Carbon monoxide is an important signaling molecule which is produced by heme oxygenase-1. CO shows antiproliferative activity against cancer cells; hence, activation of HO-1 is a significant inhibition strategy against tumor formation and survival of cancer cells. In this work, manganese-based CO-releasing molecules (CORMs) were designed and synthesized to inhibit breast cancer cell proliferation. Human invasive ductal breast cancer cells (MCF-7) were treated with the synthesized CORMs to investigate the effect of the complexes on breast cancer survival under UV light. In vitro experiments indicated that the complexes inhibited breast cancer cell proliferation, and further, the antiproliferative effects were increased under UV light. Thus, these novel CORMs may provide a drug template for the treatment of invasive ductal breast cancer.

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✉ Elvan Üstün
elvanustun77@gmail.com

[1] Department of Chemistry, Faculty of Art and Science, Ordu University, 52200 Ordu, Turkey

[2] Department of Bioengineering, Faculty of Natural Sciences and Engineering, Gaziosmanpaşa University, 60100 Tokat, Turkey

[3] Department of Chemistry, Faculty of Science, İnönü University, 44280 Malatya, Turkey

[4] Division of Biochemistry, Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Cumhuriyet University, 58140 Sivas, Turkey

Introduction

Carbon monoxide is a colorless and odorless gas that, when inhaled, enters the bloodstream and replaces the oxygen on hemoglobin to form carboxyhemoglobin and is therefore a threat to human health. Notwithstanding, CO is endogenously produced during cellular metabolism, primarily from the degradation of heme by the heme oxygenase (HO) enzyme system [1, 2]. Although CO was long thought to be only a waste product of this process, it is now widely accepted that CO is formed endogenously for cytoprotection against tissue injury/dysfunction [3, 4].

HO catalyzes the rate-limiting step in heme degradation, leading to the generation of equimolar amounts of iron, biliverdin and CO [1, 2]. HO not only regulates important biological processes including oxidative stress, inflammation, apoptosis, cell proliferation, fibrosis and angiogenesis, but also has antiinflammatory, antioxidant and antiapoptotic effects [5–7]. HO exists in two distinct isoforms, an inducible (HO-1) and a constitutive form (HO-2) [8]. The role of HO-1 in angiogenesis was first analyzed by Abraham et al. [9], who showed that overexpression of HO-1 in endothelial cells enhances their proliferation and confirmed that HO-1 promotes endothelial cell cycle progression. HO-1 was also found to protect against neuron damage, inflammation, atherosclerosis, and cardiovascular diseases [10, 11]. It is crucial for wound healing and neovascularization of ischemic heart and peripheral muscles, but can have obvious detrimental results in diseases in which angiogenesis is not desirable, such as cancer [12–16]. High levels of HO-1 may promote tumor cell survival, hindering the effectiveness of anticancer therapies; conversely, inhibition of HO has been shown to enhance tumor regression in animal models, suggesting that the HO-1 pathway may be a therapeutic target in carcinogenesis [12–20]. These

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HO-1-dependent processes are due, at least in part, to CO. The beneficial effects of CO have been demonstrated in cell culture and animal models of some diseases [4, 21–23]. Several studies have demonstrated the antitumor activity of CO, as a byproduct of HO-1. Dulak et al. [3, 4] have reported that CO inhibits proliferation of human pancreatic cancer cell lines in a dose-dependent manner. Also, Schatzschneider et al. [24] have analyzed photoinitiated cytotoxicity against the human colon cancer cell line HT29 by the complex $[Mn(CO)_3(tpm)]PF_6$. Lee et al. [25] have found that treatment with $[Ru(CO)_3Cl_2]_2$, a CO-releasing molecule (CORM), reduced the growth of human MCF-7 and MDA-MB-231 breast cancer cells.

Organometallic compounds can target DNA and protein macromolecules specifically in tumors, so reducing side effects [26]. Organometallic compounds of Mn were synthesized and tested in vitro through cell cytotoxicity and myoglobin assay in order to elucidate novel CORMs [27].

A fundamental consideration with CO-releasing systems is safe and controlled delivery of CO for therapeutic applications. This issue can be resolved by employing a photoinduced releasing system that allows one to control the location, timing and dosage of the therapeutic agent. These kinds of dark–stable, light-sensitive and potentially CO-releasing complexes are called photoCORMs (photoactivatable CO-releasing molecules). Since the first photoCORM, namely $[Mn_2(CO)_{10}]$, was reported by Motterlini et al. [28], several research groups have exploited this strategy. Schatzschneider et al. [29, 30] have successfully developed $[Mn(CO)_3(R\text{-}tpm)]^+$ complexes [tpm = tris(pyrazolyl)methane], coupled to target molecules such as peptides and SiO_2 nanoparticles, without altering the photochemical CO-release properties of the metal complexes.

Research into new CORMs has attracted considerable interest due to the favorable results obtained to date. In this study, we synthesized $[Mn(CO)_3(bpy)L]X$-type CORMs [bpy = 2,2-bipyridyl, X = PF_6 or trifluoromethanesulfonate (OTf); L = *N*-benzylbenzimidazole, *N*-(4-methylbenzyl)benzimidazole, *N*-(2,4,6-trimethylbenzyl)benzimidazole, *N*-(2,3,5,6-tetramethylbenzyl)benzimidazole or *N*-(2,3,4,5,6-pentamethylbenzyl) benzimidazole] and characterized these complexes by ^{1}H NMR, ^{13}C NMR, IR and LC–MS. The CO-releasing properties of the complexes were analyzed by myoglobin assays, and their anticancer activities were tested on human invasive ductal breast cancer cell line (MCF-7) using 5-fluorouracil (5-FU) as a control. Our results suggest that these complexes are promising candidate CORMs in cancer treatment.

Results and discussion

Synthesis and characterization

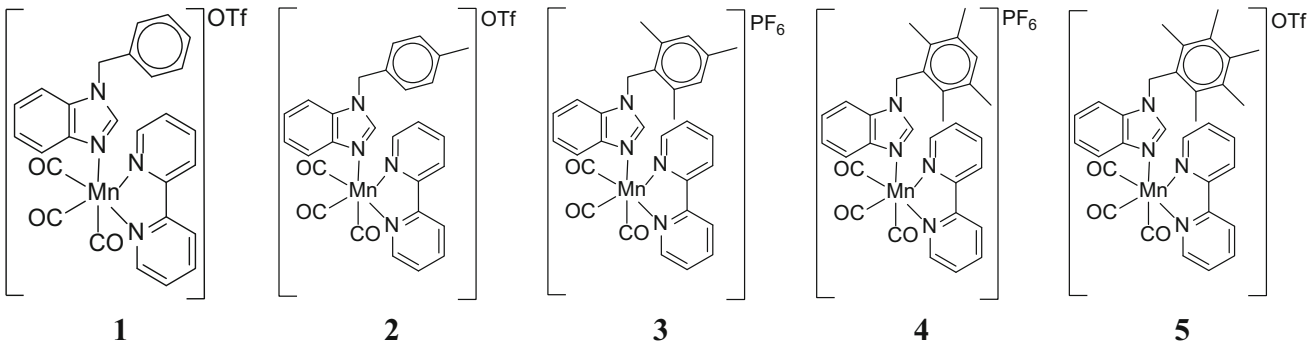
The free ligands used to synthesize these complexes (**L1**–**L5**) were characterized by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis, as detailed in the Experimental section. The NCHN hydrogens of the free ligands show singlets between 8.02 and 7.40 ppm in the ^{1}H NMR, while the NCHN carbons show resonances between 143.2 and 144.2 ppm in the ^{13}C NMR spectra. The benzyl CH_2 signal is seen between 5.25 and 5.38 ppm in the ^{1}H NMR and 43.1–48.9 ppm in the ^{13}C NMR.

The complexes were synthesized by stepwise ligand addition to pre-synthesized $Mn(CO)_3(bpy)Br$ (Scheme 1). The complexes were characterized by ^{1}H NMR, ^{13}C NMR, IR and LC–MS. In the ^{1}H NMR spectrum of complex **3**, signals at 7.71 ppm (t), 8.27 ppm (t), 8.53 ppm (d) and 9.30 ppm (d) with the expected multiplicities and integrals are assigned to bpy. A singlet at 6.52 ppm is assigned to the hydrogen atom situated between the nitrogen of the benzimidazole moiety in complex **3** (Fig. S1). A ^{1}H COSY NMR experiment was carried out on complex **3** to help in the assignment of the proton chemical shifts. The sets of cross peaks of the bipyridyl and benzimidazole derivative ligands can clearly be observed (Fig. S2). The ^{1}H NMR spectra of the other complexes were similar, as detailed in the Experimental section.

NCHN signals between 159.4 and 112.5 ppm in the ^{13}C NMR spectra of the complexes are very expected for the aromatic groups. The benzyl CH_2 groups of the ligands show signals between 55.0 and 43.5 ppm (Fig. S3 for **3**). The CH_3 substituents on the benzyl groups of the benzimidazole are observed at 55.1 ppm for **2**; 18.8 and 20.6 ppm for **3**; 14.5 and 20.0 ppm for **4**; and 15.5, 16.6 and 16.9 ppm for **5**. The ^{13}C NMR data are also detailed in the Experimental section.

IR spectra of both possible stereoisomers of the complexes should have three CO bands due to the C_S point group. The IR spectra of complexes **1** and **4** are compatible with this prediction, but the unobserved third band of complexes **2**, **3** and **5** could be obscured by the second broad carbonyl band (Fig. S4). Bands at 1604 s cm^{-1} for benzimidazoles are assigned to C–N. Features at 1261, 1229, 1146 and 1030 cm^{-1} are assigned to OTf in complexes **1**, **2** and **5**, while bands at 829 and 822 cm^{-1} are assigned to P-F stretching frequencies for complexes **3** and **4**, respectively.

The spectra of all of the complexes LC–MS were consistent with expectations. The strongest peaks are attributed to M-PF_6 and to M-OTf species.



Scheme 1 Fac-manganese(I) tricarbonyl bipyridyl complexes with benzimidazole derivative ligands

All of the complexes displayed broad maxima in the 374–379 nm range and the extinction coefficients were calculated according to the Lambert–Beer Law. Additionally, the complexes except **3** and **4** gave weak shoulders at 320–323 and 277–285 nm, whose extinction coefficients were also calculated (Table S1).

Myoglobin assays

CO-release from a coordination complex can be realized in several ways [28, 29]. Photoinduced CO-release from dark–stable metal–carbonyl complex prodrugs, as used in this study, is an important option, since many metal carbonyl complexes are sensitive to UV–visible light [30–34]. Complexes **1–5** were first dissolved in DMSO, and the solutions were kept in the absence of light for 4 h to confirm their stability. They were then irradiated with a 366-nm UV lamp in 10-min intervals. Their absorptions were measured at selected wavelengths (510 nm, isobestic point; 540 nm, Mb–CO; 557 nm, deoxy-Mb; 577 nm, Mb–CO) in the dark for 16 h (960 min) with solutions of the complexes [15 μM in 0.1 M PBS at pH = 7.4 in the presence of myoglobin (60 μM) and sodium dithionite (10 mM)] under argon atmosphere. All five complexes showed good dark stability over 16 h, with only negligible spectral changes.

Myoglobin assay is principally based on following the transformation of deoxymyoglobin to carbonmonoxy-myoglobin after the addition of CORM, using UV–Vis spectroscopy. Carbonmonoxy-myoglobin has two absorption maxima at 540 and 577 nm, while deoxymyoglobin has only one maximum at 557 nm. Hence, release of CO can be quantified using these spectral changes (Fig. S5). It is also known that irradiation of MbFe(II) under the same conditions without the manganese tricarbonyl complex in the dark does not lead to any spectral changes [26].

Total released CO, CO equivalents and half-life ($t_{1/2}$) were determined by UV–visible spectroscopy at 1-min intervals with a 366-nm UV lamp. The $t_{1/2}$ in this study is defined as the time taken to release 50% of the total CO ligands present per molecule. All the myoglobin assay measurements were taken for 45 min. The CO-releasing properties of the complexes **1–5** are given in Table 1.

One of the aims of this study to gain insight into the dependence of the CO photorelease from complexes with different numbers of methyl groups on the ligands. We conceived that boosting electron donation through increasing numbers of methyl groups could induce electron density on the metal and so strengthen Mn–CO π-back-bonding. However, complex **1** released 1.4 of 3 carbonyls (47%), while complex **5** which has five methyl substituents released 2.2 of 3 carbonyls (73%). With increasing numbers of methyl substituents on the benzyl moiety of the benzimidazole ligand, both the equivalent of released CO and MbCO concentration also increase (Table 1). These results can be explained by the stabilities of the product complexes with fewer carbonyl ligands. On the other hand, if the CO-releasing properties are considered according to half-life, there is no steady alteration depending on the number of methyl substituents (Table 1).

Cell proliferation assays

The antiproliferative activities of the complexes against the MCF-7 cell line were assayed by XTT cell proliferation. The complexes and 5-FU were incubated at different concentrations (200, 100, 50, 25, 12.5 and 6.125 μM) with breast cancer cells for 24 h in the dark. MCF-7 cells were treated with the compounds and irradiated with UV light at 366 nm for 10 min. The complexes were dissolved in DMSO and diluted in Dulbecco's modified Eagle's medium (DMEM). Control cells were treated with DMEM containing 0.1% DMSO. All five CORMs demonstrated effective anticancer activities, and the IC_{50} values of the complexes and 5-FU as control are given in Table 2.

The in vitro results indicated that these CORMs have significant anticancer potency and were able to kill MCF-7 cells in the absence of UV light. Moreover, breast cancer cell viability was found to decrease with photoactivation.

Table 1 CO-releasing data for complexes 1–5

Complexes	Concentration of MbCO (μM)	Half-life $t_{1/2}$ (min)	Equivalent of CO released
1	18.6	9.5	1.4
2	20.2	11.4	1.4
3	23.0	13.9	1.5
4	25.8	3.9	1.7
5	32.9	8.7	2.2

Table 2 IC_{50} values of CORMs and 5-FU against MCF-7 cell line

Complexes	IC_{50} (μM)	IC_{50}/UV (μM)
5-FU	13.25	9.45
1	51.93	2.91
2	22.89	12.25
3	3.22	1.79
4	17.32	1.43
5	6.49	<1

In general, the number of methyl substituents on the benzene ring of the benzimidazole skeleton significantly enhanced the anticancer and CO-releasing activities of these complexes, especially under conditions of photoactivation. Complex **3** had the lowest IC_{50} values, which can be attributed to the number of methyl groups and associated steric effects. In general, CO-release is linearly correlated to UV-treated MCF-7 cell line cytotoxicity; although complex **2** is an exception to this correlation. It is possible that the para-substituent in complex **2** may perturb electron distribution adversely with respect to UV irradiation. Nevertheless, complex **2** displayed similar cytotoxic effects to 5-FU, while the other four complexes all show better activities than 5-FU.

Conclusions

We have synthesized and characterized five manganese carbonyl complexes of general formula $Mn(CO)_3(bpy)L$, with benzimidazole derivative ligands. Our studies into the CO-releasing and anticancer activities of these manganese CORMs gave promising results, and suggest that they may find applications in the effective treatment of invasive ductal breast carcinoma.

Experimental section

Materials and methods

All reactions were carried out under argon using standard Schlenk and vacuum techniques. Solvents were freshly distilled after refluxing over metallic sodium or phosphorous pentoxide for 3–4 days. IR spectra were recorded on solid samples with a Shimadzu IRAffinity-1 ATR spectrometer. Band intensities are marked as strong (s), medium (m), weak (w) or shoulder (sh). NMR spectra were recorded on a Bruker Ultra Shield 300 MHz spectrometer. Chemical shifts δ in ppm indicate a downfield shift relative to tetramethylsilane (TMS) and were referenced relative to the solvent signals. Coupling constants J are given in Hertz. Absorption spectra were measured using a Shimadzu UV-1800 spectrophotometer equipped with quartz cuvettes ($d = 1$ cm). Elemental analyzes (C, H and N) were obtained using a CHNS-932 (LECO) instrument. LC–MS was carried out on an Agilent 1100 Series instrument. All chemicals were purchased from Sigma-Aldrich and used without further purification. The MCF-7 cell line was obtained from the American Type Culture Collection, USA. Dulbecco's modified Eagle's medium (DMEM) was obtained from Merck. Heat-inactivated fetal bovine serum was obtained from Life Technologies. Trypsin–EDTA, L-glutamine–penicillin–streptomycin and phosphate-buffered saline (PBS) solutions were obtained from Sigma-Aldrich. The XTT cell proliferation kit was purchased from Applied Chem.

Myoglobin assay

Stock solutions of the complexes for myoglobin assays were prepared in DMSO. PBS (0.1 M, pH = 7.4), 100 mM sodium dithionite (100 μL), 15 μM carbonyl complex and 60 μM myoglobin were combined in a cuvette to give a total volume of 1000 μL. Solutions were degassed by bubbling with argon at each step of the procedure. Horse muscle myoglobin solution prepared in PBS (0.1 M, pH = 7.4) was reduced to deoxymyoglobin by addition of a solution of sodium dithionite in PBS (0.1 M, pH = 7.4). Irradiation was made with a 365-nm CAMAG UV lamp at 1-min intervals during the initial 20 min and then continued at 5-min intervals until no further difference in MbCO concentration was observed. The final solution was placed 5 cm in front of the UV lamp. All irradiation experiments were carried out in triplicate. Solutions were freshly prepared for the dark stability and photoactivation experiments. Dark stability spectra were collected automatically

for the required period of time by the spectrometer software.

Preparation of L1–L5

Benzimidazole (10 mmol) was added dropwise to a solution of dried sodium hydride (10 mmol) in THF (50 mL) in an air-evacuated Schlenk flask. When gas outflow finished, the required alkyl chloride (10.1 mmol) (benzyl chloride for **L1**; 4-methylbenzyl chloride for **L2**; 2,4,6-trimethylbenzyl chloride for **L3**; 2,3,5,6-tetramethylbenzyl chloride for **L4**; and 2,3,4,5,6-pentamethylbenzyl chloride for **L5**) was added. The final solution was stirred for a day at room temperature and then refluxed for 3 h at 70 °C. Solvent was then evaporated under vacuum. The pure benzimidazole derivative ligand was obtained upon recrystallization from CH_2Cl_2/Et_2O.

N-benzylbenzimidazole (L1)

Yield: 1.98 g (95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.02 (s, 1H, NC*H*N), 7.86 (d, 1H, J = 7.2 Hz NC_6H_4N), 7.19–7.40 (m, 8H, NC_6H_4N and $CH_2C_6H_5$), 5.38 (s, 2H, $CH_2C_6H_5$). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ (ppm) = 48.9 ($CH_2C_6H_5$), 143.7 (NCHN), 143.2, 35.4, 33.9, 129.1, 128.3, 127.1, 123.2, 122.4, 120.3, 110.1 (NC_6H_4N and $CH_2C_6H_5$). Anal. Calc. for $C_{14}H_{12}N_2$ (208.26): C, 80.74; H, 5.81; N, 13.45. Found: C, 80.78; H, 5.78; N, 13.48%.

N-(4-methylbenzylbenzimidazole) (L2)

Yield: 2.05 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.84 (s, 1H, NCHN), 7.75–7.78 (m, 1H, NC_6H_4N), 6.85–7.25 (m, 7H, NC_6H_4N and $CH_2C_6H_4CH_3$), 5.26 (s, 2H, $CH_2C_6H_4CH_3$), 2.23 (s, 3H, $CH_2C_6H_4CH_3$). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ (ppm) = 48.9 ($CH_2C_6H_5$), 143.2 (NCHN), 110.6, 114.8, 119.3, 123.5, 123.9, 125.9, 126.7, 128.1, 128.8, 131.0, 132.3, 136.2 (NC_6H_4N and $CH_2C_6H_4CH_3$), 21.4 ($CH_2C_6H_4CH_3$). Anal. Calc. for $C_{15}H_{14}N_2$ (222.29): C, 81.05; H, 6.35; N, 12.60. Found: C, 81.00; H, 6.33; N, 12.67%.

N-(2,4,6-trimethylbenzylbenzimidazole) (L3)

Yield: 2.06 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.43 (s, 1H, NCHN), 7.29–7.35 (m, 2H, NC_6H_4N), 7.45–7.47 (m, 1H, NC_6H_4N), 7.81–7.83 (m, 1H, NC_6H_4N) 6.97 (s, 2H, $CH_2C_6H_2(CH_3)_3$), 5.25 (s, 2H, $CH_2C_6H_2(CH_3)_3$), 2.34 and 2.25 (s, 9H, $CH_2C_6H_2(CH_3)_3$). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ (ppm) = 43.1 ($CH_2C_6H_2(CH_3)_3$), 144.1 (NCHN), 141.7, 134.2, 122.8, 122.2, 120.4, and 109.5 (NC_6H_4N), 138.8, 137.8, 129.6 and127.1 ($CH_2C_6H_2(CH_3)_3$), 19.5 and 21.0 ($CH_2C_6H_2(CH_3)_3$). Anal. Calc. for $C_{17}H_{18}N_2$ (250.34): C, 81.56; H, 7.25; N, 11.19. Found: C, 81.60; H, 7.27; N, 11.14%.

N-(2,3,5,6-tetramethylbenzylbenzimidazole) (L4)

Yield: 2.43 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.45 (s, 1H, NCHN), 7.88–7.84 (m, 1H, NC_6H_4N), 7.58–7.55 (m, 1H, NC_6H_4N), 7.43–7.33 (m, 2H, NC_6H_4N), 7.11 (s, 1H, $CH_2C_6H(CH_3)_4$), 5.33 (s, 2H, $CH_2C_6H(CH_3)_4$), 2.31 and 2.18 (s, 12H, $CH_2C_6H(CH_3)_4$-2,3,5,6). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ (ppm) = 43.9 ($CH_2C_6H(CH_3)_4$), 143.2 (NCHN), 141.5, 133.9, 122.6, 120.1, 113.7 and 109.7 (NC_6H_4N), 134.7, 132.7, 129.6 and 123.1 ($CH_2C_6H(CH_3)_4$), 20.5 and 15.5 ($CH_2C_6H(CH_3)_4$). Anal. Calc. for $C_{18}H_{20}N_2$ (264.36): C, 81.78; H, 7.63; N, 10.60. Found: C, 81.75; H, 7.67; N, 10.57%.

N-(2,3,4,5,6-pentamethylbenzylbenzimidazole) (L5)

Yield: 2.39 g (86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.40 (s, 1H, NCHN), 7.30–7.37 (m, 2H, NC_6H_4N), 7.52–7.54 (m, 1H, NC_6H_4N), 7.82–7.84 (m, 1H, NC_6H_4N), 5.31 (s, 2H, $CH_2C_6(CH_3)_5$), 2.21, 2.27 and 2.32 (s, 15H, $CH_2C_6(CH_3)_5$). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ (ppm) = 144.2 (NCHN), 141.9, 134.1, 133.4, 122.2, 120.4 and 109.5 (NC_6H_4N), 136.2, 133.5 and 122.7 ($CH_2C_6(CH_3)_5$), 44.3 ($CH_2C_6(CH_3)_5$), 16.5, 16.8 and 17.2 ($CH_2C_6(CH_3)_5$). Anal. Calc. for $C_{14}H_{12}N_2$ (278.39): C, 81.97; H, 7.97; N, 10.06. Found: C, 81.94; H, 7.92; N, 10.01%.

Preparation of complexes 1–5

The complexes were prepared by similar methods. $Mn(CO)_3$(bpy)Br (100 mg, 0.27 mmol) was added to a solution of AgOTf (82.2 mg, 0.320 mmol) in acetone (10 mL). Precipitated AgBr was filtered out with celite, and the ligand (0.32 mmol) was added to the filtrate after stirring for a day at room temperature. Acetone was evaporated under vacuum, and KPF_6/methanol solution (10 mL) was added. The resulting orange precipitate was filtered off and washed with 5 mL cold methanol followed by 10 mL cold diethyl ether. There was no need to change anion for **1**, **2** and **5**; these complexes precipitated directly and were filtered off and washed with 5 mL cold methanol followed by 10 mL cold diethyl ether. At all steps of the synthesis, all glassware was protected from light by wrapping in aluminum foil.

[Mn(CO)$_3$(bpy)(L1)]OTf (1)

Yield: 136 mg (78%). ^{1}H NMR (300 MHz, DMSO-D_6): δ (ppm) = 5.36 (s, 2H, $NCH_2C_6H_5$), 9.48 (s, 1H, NCHN),

8.60–6.92 (m, 16H, $NCH_2C_6H_5$, $NC_{10}H_8N$ and $N_2C_6H_5$). ^{13}C NMR (300 MHz, DMSO-D_6): δ (ppm) = 47.9 ($NCH_2C_6H_5$), 145.9 (NCHN), 155.0, 140.4, 128.7, 127.8, 127.1 ($N_2C_{10}H_8$), 154.8, 141.3, 135.4, 133.0 ($NCH_2C_6H_5$), 128.0, 124.0, 117.1, 112.5 (NC_6H_4N). LCMS: *m/z* 503.1 $[M\text{-}OTf]^+$. IR (cm^{-1}, ATR): ν = 1442.7, 1519.9 (s, C-H), 1604.8 (s, C–N), 2033.0, 1944.2, 1925.0 (s, CO).

[Mn(CO)$_3$(bpy)(L2)]OTf (2)

Yield: 145 mg (82%). ^1H NMR (300 MHz, DMSO-D_6) δ (ppm) = 5.68 (s, 2H, $NCH_2C_4H_4Cl$), 3.75 (s, 3H, $NCH_2C_4H_4CH_3$), 9.49 (d, J = 4.0, 2H, $NCH_2C_4H_4CH_3$), 8.60 (d, *J* = 6.0, 2H, $NCH_2C_4H_4CH_3$), 7.30–6.79 (m, 6H, NC_6H_4N and $NC_{10}H_8N$), 7.99 (s, 1H, NCHN), 8.32 (t, *J* = 6.5, 2H, $NC_{10}H_8N$), 7.84 (t, *J* = 5.5, $NC_{10}H_8N$), 7.79 (d, *J* = 4.0, $NC_{10}H_8N$). ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm) = 49.5 ($NCH_2C_6H_4CH_3$), 55.1 ($NCH_2C_6H_4CH_3$), 159.4 (N*C*HN), 154.9, 140.3, 130.9, 130.0, 128.7 ($N_2C_{10}H_8$), 154.7, 142.0, 132.8, 127.7, 124. 0 ($NCH_2C_6H_4CH_3$), 126.6, 125.5, 114.3, 113.9 (NC_6H_4N). LCMS: *m/z* 517.4 $[M\text{-}OTf]^+$. IR (cm^{-1}, ATR): 1442.6, 1473.6 (s, C–H), 1604.8 (s, C–N), 2040.7, 1932.7 (s, CO).

[Mn(CO)$_3$(bpy)(L3)]PF$_6$ (3)

Yield: 141 mg (76%). ^1H NMR (300 MHz, DMSO-D_6) δ (ppm) = 1.76 (s, 6H, $NCH_2C_6H_2(CH_3)_3$), 2.33 (s, 3H, $NCH_2C_6H_2(CH_3)_3$), 7.43–7.36 (m, 2H, NC_6H_4N), 7.53 (d, *J* = 8.0, NC_6H_4N), 7.93 (d, *J* = 8.0, NC_6H_4N), 5.23 (s, 2H, $NCH_2C_6H_2(CH_3)_3$), 6.52 (s, 1H, NCHN), 6.62 (s, 2H, $NCH_2C_6H_2(CH_3)_3$), 7.71 (t, *J* = 7.0, 2H, $NC_{10}H_8N$, 5/5′), 8,27 (t, *J* = 9.0, 2H, $NC_{10}H_8N$, 4/4′), 8,53 (d, *J* = 8.0, 2H, $NC_{10}H_8N$, 3/3′), 9,30 (d, *J* = 5.5, 2H, $NC_{10}H_8N$, 6/6′). ^{13}C NMR (300 MHz, DMSO-D_6) δ (ppm) = 18.7 ($NCH_2C_6H_2(CH_3)_3$), 20.5 ($NCH_2C_6H_2(CH_3)_3$), 43.5 ($NCH_2C_6H_2(CH_3)_3$), 112.3 (NCHN), 143.3, 141.7, 138.1, 137.0, 133.4, 129.2, 127.6, 126.4, 124.2, 123.9, 123.9, 117.1 ($NCH_2C_6H_2(CH_3)_3$), 154.6, 154.3, 140.3, 137.4, 129. 3 ($NC_{10}H_8N$). LCMS: *m/z* 545.1 $[M\text{-}PF6]^+$. IR (cm^{-1}, ATR): 1446.6, 1473.6 (s, C–H), 1600.9 (s, C–N), 2036.8, 1952.0, 1928.8 (s, CO).

[Mn(CO)$_3$(bpy)(L4)]PF$_6$ (4)

Yield: 164 mg (87%). ^1H NMR (300 MHz, DMSO-D_6) δ (ppm) = 1.66 (s, 6H, $NCH_2C_6H(CH_3)_4$), 2.18 (s, 6H, $NCH_2C_6H(CH_3)_4$), 5.28 (s, 2H, $NCH_2C_6H(CH_3)_4$), 6.12 (s, 1H, NCHN), 7.07 (s, 1H, NC_6H_4N), 7.48–7.67 (m, 2H, $NC_{10}H_8N$), 7.71–7.67 (m, 3H, NC_6H_4N), 7.98 (d, *J* = 8.4, 1H, NC_6H_4N), 8.26 (t, *J* = 7.8, 2H, $NC_{10}H_8N$), 8.51 (d, *J* = 7.8, 2H, $NC_{10}H_8N$), 9.22 (d, *J* = 4.8, 2H, $NC_{10}H_8N$). ^{13}C NMR (300 MHz, DMSO-D_6) δ (ppm) = 14.5, 20.0 ($NCH_2C_6H(CH_3)_4$), 54.9 ($NCH_2C_6H(CH_3)_4$), 112.5 (NCHN), 142.5, 141.8, 133.9, 133.6, 133.1, ($NCH_2C_6H(CH_3)_4$), 132.3, 129.0, 124.3, 124.2 (NC_6H_4N), 154.5, 154.3, 140.4, 127.7, 123.7 ($NC_{10}H_8N$). LCMS: *m/z* 559.2 $[M\text{-}PF_6]^+$. IR (cm^{-1}, ATR): *v* = 1446.6, 1516.0 (s, C–H), 1608.6 (s, C–N), 2036.8, 1932.7 (s, CO).

[Mn(CO)$_3$(bpy)(L5)]OTf (5)

Yield: 166 mg (86%). ^1H NMR (300 MHz, DMSO-D_6) δ (ppm) = 1.66 (s, 6H, $NCH_2C_6H_2(CH_3)_5$), 2.15 (s, 6H, $NCH_2C_6H_2(CH_3)_5$), 2.34 (s, 3H, $NCH_2C_6H_2(CH_3)_3$), 5.24 (s, 2H, $NCH_2C_6H_2(CH_3)_3$), 5.89 (s, 1H, NCHN), 7.73-7.64 (m, 4H, NC_6H_4N), 8.25 (t, *J* = 7.4, 2H, $NC_{10}H_8N$, 5/5′), 7.54–7.42 (m, 2H, $NC_{10}H_8N$, 4/4′), 8,48 (d, J = 7.8, 2H, $NC_{10}H_8N$, 3/3′), 9,17 (d, J = 5.2, 2H, $NC_{10}H_8N$, 6/6′). ^{13}C NMR (300 MHz, DMSO-D_6) δ (ppm) = 15.5 ($NCH_2C_6(CH_3)_5$), 16.6 ($NCH_2C_6(CH_3)_5$), 16.9 ($NCH_2C_6(CH_3)_5$), 112.6 (NCHN), 117.2 ($NCH_2C_6(CH_3)_5$) 135.8, 135.1, 133.6, 132.7, 132.6, 132.5 ($NCH_2C_6(CH_3)_3$), 142.1, 142.0, 126.2, 124.4, 124.3 (NC_6H_4N) 154.5, 154.3, 140.3, 127.7, 123.7 ($NC_{10}H_8N$). LCMS: *m/z* 573.3 $[M\text{-}OTf]^+$. IR (cm^{-1}, ATR): 1446.6, 1473.6 (s, C–H), 1608.6 (s, C–N), 2033.0, 1936.5 (s, CO).

Cell culture and growth

MCF-7 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, 100 IU mL^{-1} penicillin and 10 mg mL^{-1} streptomycin in 75 cm^2 polystyrene flasks. Cells were cultivated at 37 °C in a humidified atmosphere with 5% CO_2.

XTT cell proliferation assay

MCF-7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS (heat-inactivated fetal bovine serum), 1% L-glutamine, 100 IU mL^{-1} penicillin and 10 mg mL^{-1} streptomycin in 75 cm^2 polystyrene flasks. Cells were cultivated at 37 °C in a humidified atmosphere with 5% CO_2.

The anticancer activities of the manganese CORMs were determined using the 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) cell proliferation assay. Approximately 10×10^4 MCF-7 cells (final volume of 200 µl) were seeded in 96-well flat-bottom ELISA plates and incubated overnight at 37 °C in a 5% CO_2 incubator. Wells were treated with different concentrations of the complexes and then incubated at 37 °C in 5% CO_2 for 48 h. XTT solution was then applied to each well and the plates were incubated at 37 °C for 4 h. Optical densities of the plates was measured using an ELISA reader at 450 nm.

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