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Original article

Bioactivity of novel transition metal complexes of *N*′-[(4-methoxy)thiobenzoyl]benzoic acid hydrazide

Anuraag Shrivastav [a,b], Pratibha Tripathi [c], Ajay K. Srivastava [c], Nand K. Singh [c,**], Rajendra K. Sharma [a,b,*]

[a] *Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan, 20 Campus Drive, Saskatoon, SK, Canada S7N 4H4*
[b] *Saskatchewan Cancer Agency, Saskatoon, SK, Canada S7N 4H4*
[c] *Department of Chemistry, Banaras Hindu University, Varanasi 221005, India*

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Abstract

Cu(II), Fe(III), and Mn(II) complexes of a novel ligand *N*′-[(4-methoxy)thiobenzoyl]benzoic acid hydrazide (H_2mtbh) have been synthesized and characterized by elemental analyses, IR, UV–vis, NMR, mass, EPR and Mössbauer spectroscopy. The results suggest a square planar structure for [Cu(Hmtbh)Cl] and [Cu(mtbh)] whereas an octahedral structure for [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)]. Mn(II) and Fe(III) complexes were found to inhibit proliferation of HT29 cells. [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)] inhibited proliferation of HT29 cells with half maximal inhibition (IC_{50}) of 8.15 ± 0.87 and 68.1 ± 4.8 μM, respectively, whereas H_2mtbh showed growth inhibition with IC_{50} of 90.9 ± 7.8 μM and were able to inhibit NMT activity in vitro. Mn(II) and Fe(III) complexes inhibited NMT activity in a dose dependent manner with IC_{50} values of 20 ± 2.2 and 60 ± 7.2 μM, respectively, whereas ligand (H_2mtbh) displayed IC_{50} of 3.2 ± 0.5 mM.

Keywords: Cytotoxic activity; Transition metal complexes; *N*-Myristoyltransferase

1. Introduction

The study of biologically important compounds has led to the development of several chemotherapeutic drugs in past few decades, the most notable example of which is cisplatin. Since cisplatin emerged as the most important anticancer drug [1,2] number of coordination compounds of other transition metal ions have been synthesized and characterized [3,4] to study the effect of metal, ligand and other substituents on structural and kinetic properties involved in biological activity [5,6]. However, significant problems are still extant including side effects, toxicity, cancer specificity and specially acquired resistance. The toxicity associated with platinum drugs limits its extensive use in the treatment of cancer. These facts provoke the search for a new drug with a different mode of action [7,8]. Earlier transition metals are less toxic and have shown promising antitumor activity [9–13]. These challenges are exemplified by the problems encountered in the use of general anticancer drugs. Recently, *N*-myristoyltransferase has emerged as a novel therapeutic target for cancer. N-myristoylation is referred to the addition of myristic acid to the N-terminal glycine residue of the protein [14,15]. Many proteins involved in a wide variety of a signal cascade, cellular transformations and oncogenesis are myristoylated [14–16]. Examples include the catalytic subunit of cAMP-dependent protein kinase [17], the β-subunit of calcineurin [18], the α-subunit of several G-proteins [19], the cellular and transforming

* Corresponding author. Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan, 20 Campus Drive, Saskatoon, SK, Canada S7N 4H4. Tel.: +1 306 966 7733; fax: +1 306 655 2635.
** Corresponding author. Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, UP, India. Tel.:+91 542 2307321; fax: +91 542 2368174.
E-mail addresses: nksingh@bhu.ac.in (N.K. Singh), rsharma@scf.sk.ca (R.K. Sharma).

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forms of pp60^{src} [20], several tyrosine kinases and proteins important for the assembly, maturation and infectivity of mature virus particles, such as the murine leukemia virus Pr65^{gag} precursor [21] and poliovirus VPO polypeptide precursor [22].

Myristoylation of proteins is catalyzed by the eukaryotic enzyme NMT. Earlier we reported for the first time in a rat model that NMT is more active in colonic epithelial neoplasms than in the corresponding normal appearing colonic tissue, and that an increase in NMT activity appears at an early stage in colonic carcinogenesis [23]. Increased NMT activity was also observed in human colonic tumors and was predominantly cytosolic [24]. Furthermore, colorectal tumors showed increased immunohistochemical staining for NMT compared to normal mucosa [24]. In addition, gallbladder carcinoma displayed strong cytoplasmic positivity for NMT with an increased intensity in the invasive component. Normal gallbladder mucosa showed weak to negative cytoplasmic staining [25].

A large number of diverse structures and proteins are capable of inhibiting NMT [26–31]. Earlier, we have demonstrated that early transition metal complexes of sulfur and nitrogen donor ligands have dual mechanism of action in tumor regression [12]. These metal complexes have protective effect on normal cells and selectively induced apoptosis in tumor cells [12]. Therefore, we synthesized novel sulfur and nitrogen donor ligand and its metal complexes. Various ligands with a −C(S)NHNH(O)C− skeleton have been prepared in order to understand the pharmacophore responsible for anti-proliferative and anti-NMT activity. Present paper aims to study the anti-proliferative and anti-NMT activity of Cu(II), Fe(III) and Mn(II) complexes of a novel nitrogen and sulfur donor ligand *N*′-[(4-methoxy)-thiobenzoyl]benzoic acid hydrazide (H_2mtbh) (Fig. 1).

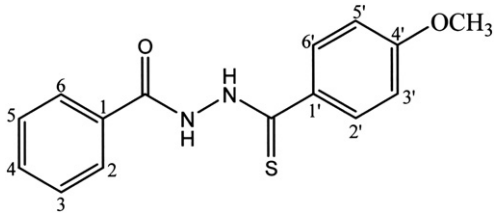

Fig. 1. Tentative structure of the ligand *N*′-[(4-methoxy)thiobenzoyl]benzoic acid hydrazide (H_2mtbh).

2. Results and discussion

2.1. Chemistry

Elemental analyses reveal that H_2mtbh and its complexes are of good quality. From the analytical data it is clear that H_2mtbh reacts with metal salts either in 1:1 or 2:1 molar proportion by losing either one or both the hydrazinic protons from each ligand. The complexes are soluble in polar coordinating solvents such as DMSO and are colored. The formation of the complexes is given below:

$$CuCl_2\cdot 2H_2O + H_2mtbh \rightarrow [Cu(Hmtbh)Cl] + HCl + 2H_2O$$

$$Cu(OAc)_2\cdot H_2O + H_2mtbh \rightarrow [Cu(mtbh)] + 2AcOH + H_2O$$

$$Mn(OAc)_2\cdot 4H_2O + 2H_2mtbh \rightarrow [Mn(Hmtbh)_2] + 2AcOH + 4H_2O$$

$$[Fe(acac)_3] + 2H_2mtbh \rightarrow [Fe(Hmtbh)(mtbh)] + 3Hacac$$

The magnetic moment of 2.1 and 1.89 BM for [Cu(Hmtbh)Cl] and [Cu(mtbh)], respectively, are normal as expected for the presence of one unpaired electron. Both the complexes display a broad UV–vis band at 16 130 and 17 200 cm^{-1} assigned to the envelope of $^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{2g}$ and ^2E_g transitions and suggest a square planar geometry for Cu(II) [12]. The magnetic moment of 6.0 and 6.01 BM for [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)], respectively, suggest their high spin nature. [Mn(Hmtbh)$_2$] shows two UV–vis bands at 18 020 and 24 690 cm^{-1} assigned to the $^6A_{1g} \rightarrow {}^4T_{1g}$, $^4T_{2g}$ transitions, respectively, in an octahedral geometry around high spin Mn(II). A magnetic moment of 6.01 BM and the presence of an UV–vis band at 19 800 cm^{-1} attributed to the $^6A_{1g} \rightarrow {}^4T_{2g}$ transition is in accord with the octahedral geometry of [Fe(Hmtbh)(mtbh)] [32].

The ^{1}H NMR spectrum of H_2mtbh exhibits two signals at δ 11.9 and 11.1 ppm for the amide and thioamide protons, which disappear on D_2O exchange. The signal at δ 3.9 ppm is attributed to $-OCH_3$ protons of 4-methoxythiobenzoyl part. The protons due to the two aromatic rings appear as multiplets between δ 8.01 and 7.0 ppm. The ^{13}C NMR spectrum of H_2mtbh shows 11 signals due to 15 carbon atoms, out of which signals at δ 195.43 and 164.81 ppm are due to >C=S and >C=O carbons, respectively. The assignment of the ring carbons for 4-methoxythiobenzoyl moiety has been made by comparing the spectrum of the H_2mtbh with benzoic acid hydrazide [33].

Important IR spectral data of the ligands and complexes are summarized in Table 1. The IR spectrum of H_2mtbh shows bands at 3164 and 3105 cm^{-1} for the two −NH groups present in the ligand. The bands occurring at 1657, 1433, 1343, 902 and 833 cm^{-1} are assigned to ν(C=O), thioamide I [β(NH) + ν(CN)], thioamide II [ν(CN) + β(NH)], ν(N–N) and ν(C=S), respectively [34]. An exhaustive comparison of the IR spectra of the ligand and complexes gave information about the mode of bonding of the ligand in metal complexes. The IR spectrum of [Cu(mtbh)] when compared with H_2mtbh, indicates that bands due to ν(NH), ν(C=O) and ν(C=S) are absent, but new bands appear at ca. 1602 and 763 cm^{-1} due to ν(N=C) of NCO and ν(C–S), respectively, suggesting removal of both the hydrazinic protons via enolisation and thioenolisation and bonding of the resulting enolic oxygen and thiolato sulfur takes place with Cu(II). Furthermore, the ligand bands due to thioamide I, thioamide II and ν(N–N) undergo a positive shift of 46, 57 and 60 cm^{-1}, respectively. The magnitude of the positive shift supports that enolic oxygen,

Table 1
Important IR spectral bands (cm^{-1}) and their assignments

Assignments	[H_2mtbh]	[Cu(mtbh)]	[Cu(Hmtbh)Cl]	[Mn(Hmtbh)$_2$]	[Fe(Hmtbh)(mtbh)]
ν(NH)	3164, 3105	–	3106	3128	3123
ν(C=O)/ν(NCO)	1657	1602	1624 (−33)	1620 (−37)	1622, 1600
Thioamide(I), ν[βNH + ν(CN)]	1433	1479 (+46)	1460 (+27)	1470 (+37)	1473 (+40)
Thioamide(II), ν[(CN) + β(NH)	1343	1400	1394 (+51)	1400 (+57)	1403 (+40)
ν(N–N)	902	962 (+60)	952 (+50)	952 (+50)	952 (+50)
ν(C=S)/(C–S)	833	763	789	792	780

Figures in parentheses indicate shifts in band position.

thiolato sulfur and both hydrazinic nitrogens are involved in coordination and H_2mtbh behaves as binegatively charged quadridentate species in [Cu(mtbh)] [35]. The IR spectra of [Cu(Hmtbh)Cl] and [Mn(Hmtbh)$_2$] show a band at 3106–3128 cm^{-1} due to ν(NH), suggesting loss of either of the two hydrazinic protons upon complexation. A negative shift of 33–37 cm^{-1} in ν(C=O), the disappearance of ν(C=S) and appearance of a new band at 789–92 cm^{-1} due to ν(C–S), indicate bonding through carbonyl oxygen and thiolato sulfur. Bands due to thioamide I, thioamide II and ν(N–N) undergo positive shift of 27–37, 51–57 and 50 cm^{-1} suggesting the participation of above described sites in bonding and also either of the two hydrazinic nitrogens. The divalent metal ions Mn(II) and Cu(II) involve mononegative tridentate ligand bonding through carbonyl oxygen, thiolato sulfur and one of the two hydrazinic nitrogens. The spectrum of [Fe(Hmtbh)(mtbh)] shows a band at 3123 cm^{-1} due to ν(NH). Presence of two peaks at 1622 and 1600 cm^{-1} for ν(C=O) and ν(C=N) of NCO, respectively, indicates that one of the two ligands is bonding in the keto form while the other in enolic form. The disappearance of ν(C=S) and appearance of a new band at 780 cm^{-1} due ν(C–S) indicates bonding through thiolato sulfur. Further, a positive shift of 50 cm^{-1} in ν(N–N) suggests bonding through hydrazine nitrogen. Thus, out of the two ligands, one acts as a uninegative tridentate, bonding through thiolato sulfur, hydrazine nitrogen and carbonyl oxygen, while other acts as dinegetive tridentate, the bonding sites being thiolato sulfur, hydrazine nitrogen and enolic oxygen [12,33].

The powder EPR spectrum of [Cu(Hmtbh)Cl] obtained in the X-band region displays two peaks with $g_{\|} = 2.175$ and $g_{\perp} = 2.053$, while that of [Cu(mtbh)] shows an axial spectrum with $g_{\|} = 2.152$ and $g_{\perp} = 2.02$. The obtained spectral parameters are in agreement with square planar geometry around Cu(II). [Fe(Hmtbh)(mtbh)] shows two peaks with $g_1 = 2.076$ and $g_2 = 2.04$ as observed for a distorted octahedral Fe(III) complex.

The ligand H_2mtbh is identified by FAB mass spectrum, which shows many peaks due to various fragments. The molecular ion peak $[MH]^+$ is observed at $m/z = 287$. Other important peaks are those at $m/z = 253$ (65% intensity) and 151 (90% intensity) corresponding to fragments formed from the MH^+ after the release of H_2S and PhCONHN, respectively. FAB mass spectral analyses were carried out for the complexes [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)] which show the presence of the corresponding molecular ion peak and confirms the composition of the complexes.

The Mössbauer spectrum of [Fe(Hmtbh)(mtbh)] has been recorded at liquid nitrogen temperature. The isomer shift, $\delta = 0.047$ mm/s and quadrupole splitting, $\Delta Eq = 0.374$ mm/s supports an octahedral geometry around high spin Fe(III) and is undoubtely a single species formed by one dinegative and an uninegative ligand [36]. On the basis of above physiochemical studies the tentative structures of the complexes are given in Fig. 2.

2.2. Bio-evaluation of novel metal complexes

To investigate the antitumor activity of H_2mtbh and its metal complexes, their effect on the growth of HT29 cells in vitro was measured by MTT assay. It was observed that treatment of tumor cells with [Mn(Hmtbh)$_2$] caused maximum growth inhibition followed by [Fe(Hmtbh)(mtbh)] and the ligand. [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)] showed antiproliferative effect with half maximal inhibition (IC_{50}) of 8.15 ± 0.87 and 68.1 ± 4.8 μM, respectively, whereas H_2mtbh showed growth inhibition with IC_{50} of 90.9 ± 7.8 μM. Cu(II) complexes precipitated out while giving treatments hence its bioactivity could not be determined. The reason for the observed inhibition of tumor cell growth by the metal complexes is unclear, however, several possibilities could be considered. The cytostatic activity of the metal complexes could be a direct result of the interaction of the metal complexes with DNA, thus interfering with the process of DNA replication. Indeed, DNA binding of metal complexes has been documented [37]. Furthermore, inhibition/activation of various enzymes directly/indirectly involved in DNA replication is not ruled out. These results may also indicate a possible decline of the overall metabolic activity of the tumor cells with a concomitant inhibition of the activity of various enzymes involved in respiration. Interaction of metal complexes with protein components of viable cells has been reported [38]. Binding of metal complexes with protein may cause alterations in the structural and functional organization of proteins. Studies from various laboratories including ours have established *N*-myristoyltransferase (NMT) as a putative therapeutic target for cancer. We reported earlier increased activity and expression of NMT during the early stages of colon cancer. Very recently we had demonstrated that Cu(II) and Mn(III) complexes of *N*′-[(2-hydroxyphenyl)carbonothioyl]pyridine-2-carbohydrazide inhibited NMT activity in vitro and specifically reduced the levels of NMT2 [39]. It has been demonstrated that NMT plays a crucial role in the apoptosis [40,41]. Furthermore it has been

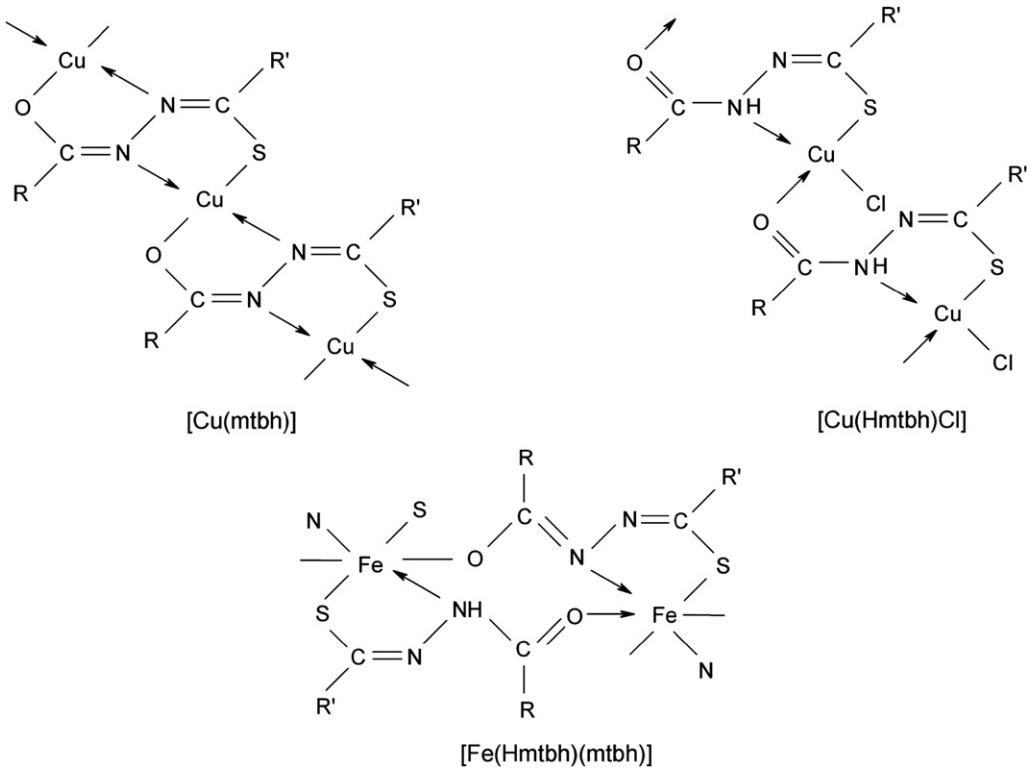

Fig. 2. Tentative structures of the Cu(II) and Fe(III) complexes.

demonstrated that ablation of NMT1 and NMT2 results in the apoptotic cell death [42]. Therefore, we also investigated the effect of these metal complexes on NMT activity. The anti-NMT activity of [Cu(mtbh)] could not be evaluated due to its precipitation in the assay reaction mixture. However, Mn(II) and Fe(III) complexes displayed anti-NMT activity in vitro. Mn(II) and Fe(III) inhibited NMT activity in a dose dependent manner with IC_{50} values of 20 ± 2.2 and 60 ± 7.2 μM, respectively (Fig. 3a and b), whereas, ligand (H_2mtbh) displayed IC_{50} of 3.2 ± 0.5 mM. These results suggest that complexation with metals increased the anti-proliferative and anti-NMT activity of the newly synthesized ligand. Increased anti-proliferative activity upon complex formation of the nitrogen and sulfur donor ligands with transition metal complexes have been reported earlier [12,13]. Transition metal complexes of nitrogen and sulfur donor ligands are emerging as a new class of NMT inhibitors. Further work is in progress to understand the interaction of metal complexes with NMT and its mode of action.

3. Experimental protocols

3.1. General materials and techniques

All chemicals of analytical grade were used after further purification. Ammonium polysulfide [43] and tris(acetylacetonato)iron(III) [44] were prepared by the reported procedures. The sodium salt of (4-methoxyphenyl)carbonothioylthioacetic acid [45] was prepared by treating a solution of 4-methoxy benzaldehyde (24 ml) in EtOH (40 ml) at 65 °C with freshly prepared ammonium polysulfide solution (135 ml). The reaction mixture was refluxed for 30 min and immediately cooled in ice. A red solution was separated from the red resinous mass and acidified with conc. HCl in the presence of Et_2O under ice-cooled condition. 4-Methoxydithio benzoic acid separated as red oil in the Et_2O layer. The remaining resinous mass was refluxed for 8 h in EtOH (40 ml) and KOH (5 g), and then cooled in ice, acidified with conc. HCl in the presence of Et_2O. Both the ethereal extracts were washed with water (3×40 ml) and then shaken with 1 N NaOH (3×50 ml) in order to extract the sodium salt of 4-methoxydithiobenzoate. To this sodium salt was added a solution of $ClCH_2COOH$ (10 g) already neutralized with sodium carbonate and maintained the pH of the solution at 7.0. After standing the mixture overnight at room temperature, it was acidified with conc. HCl to precipitate the ester, (4-methoxyphenyl)carbonothioylthioacetic acid. The orange yellow colored ester was recrystallised from MeOH in the presence of charcoal. Yield, 9 g (21%); m.p. 127 °C (lit. 122–25 °C).

3.2. Preparation of the ligand

N′-[(4-Methoxy)thiobenzoyl]benzoic acid hydrazide (H_2mtbh) was prepared by reacting (4-methoxyphenyl)carbonothioylthioacetic acid (2.42 g, 10 mmol) and benzoic acid hydrazide (BAH) (1.38 g, 10 mmol), both were dissolved separately in aqueous NaOH (1 N, 10 ml), mixed together and filtered. The mixture

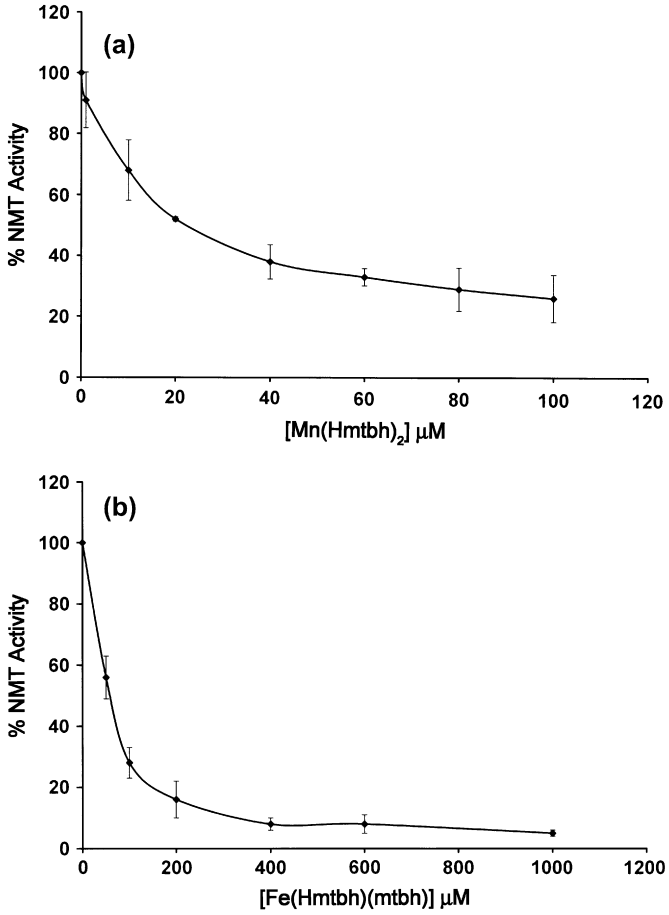

Fig. 3. Dose dependent inhibition of hNMT by $[Mn(Hmtbh)_2]$ and [Fe(Hmtbh)(mtbh)]. Purified recombinant human NMT (0.2 μg) was incubated in the presence of various concentration of (a) $[Mn(Hmtbh)_2]$ or (b) [Fe(Hmtbh)(mtbh)] and its activity was determined as described under Section 3. Values are mean of three independent experiments carried out in duplicates ± standard deviation.

was kept at room temperature for 2 h and then acidified with dil. AcOH (20%) whereupon a yellow precipitate formed. It was suction filtered, washed with water, dried in vacuo and recrystallised from MeOH. Yield, 1.5 g (52%); m. p. 138 °C. Anal. Found (%): C, 62.9; H, 4.8; N, 9.3. Calculated (%) for $C_{15}H_{14}N_2O_2S$ (286): C, 62.9; H, 4.8; N, 9.7. IR (ν cm^{-1}, KBr): 3164 m and 3105 m (N–H), 1657 s (C=O), 1433 s (thioamide I), 1343 s (thioamide II), 902 s (N–N), 833 m (C=S). ^1H NMR (DMSO-d_6; δ ppm): 3.9 (s, 3H, OMe), 7.0, 7.5, 7.6, 8.0 (multiplets, 9H, phenyl), 11.1 (s, 1H, CSN–H), 11.9 (s, 1H, CON–H). ^{13}C NMR (DMSO-d_6; δ ppm): 55.53 (OMe), 132.04 (C-1), 127.57 (C-2, C-6), 128.63 (C-3, C-5), 131.09 (C-4), 132.64 (C′-1), 129.61 (C′-2, C′-6), 113.41 (C′-3, C′-5), 161.89 (C′-4), 164.81 (C=O), 195.43 (C=S) (Fig. 2). UV–vis (ν cm^{-1}): 39 680, 35 210, 32 460. MS (FAB): $m/z = 287$ $[M+1]^+$, 269 $[M-H_2O]^+$, 253 $[M-H_2S]^+$, 176 $[M-(H_2S+Ph)]^+$, 151 (Base Peak) $[(4\text{-}CH_3O)PhCS]^+$, 105 $[PhCO]^+$. Based on the above physico-chemical studies the tentative structure of the ligand is depicted in Fig. 1.

3.3. Preparation of complexes

A solution of H_2mtbh (0.286 or 0.552 g, 1 or 2 mmol as appropriate) in $CHCl_3$ (15 cm^3) was added to a solution of $CuCl_2\cdot 2H_2O$ or $Cu(OAc)_2\cdot H_2O$ or $Mn(OAc)_2\cdot 4H_2O$ (1 mmol) in 10 cm^3 of MeOH. The mixture was magnetically stirred for 2 h at room temperature. The resulting solids were filtered off, washed thoroughly with EtOH and dried in vacuo. Yield ranged from 45 to 65%. The complexes were characterized using the techniques mentioned earlier.

[Cu(Hmtbh)Cl]: dark green. Anal. Found (%): C, 47.2; H, 3.8; N, 7.8; Cl, 9.2; Cu, 16.4. Calculated (%) for $C_{15}H_{13}ClCuN_2O_2S$ (384.1): C, 46.8; H, 3.4; N, 7.3; Cl, 9.2; Cu, 16.5. IR (ν cm^{-1}, KBr): 3106 m (N–H), 1624 s (C=O), 1468 s (thioamide I), 1394 s (thioamide II), 952 s (N–N), 786 m (C–S). UV–vis (ν cm^{-1}): 17 200, 31 250, 32 890.

[Cu(mtbh)]: black. Anal. Found (%): C, 52.0; H, 3.6; N, 8.4; Cu, 17.6. Calculated (%) for $C_{15}H_{12}CuN_2O_2S$ (348): C, 51.8; H, 3.5; N, 8.0; Cu, 18.2. IR (ν cm^{-1}, KBr): 1602 s (NCO), 1479 s (thioamide I), 1400 s (thioamide II), 962 s (N–N), 768 m (C–S). UV–vis (ν cm^{-1}): 16 130, 21 505, 26 315.

$[Mn(Hmtbh)_2]$: yellow. Anal. Found (%): C, 57.8; H, 4.3; N, 8.8; Mn, 9.3. Calculated (%) for $C_{30}H_{26}MnN_4O_4S_2$ (625.4): C, 57.6; H, 4.2; N, 8.9; Mn, 8.7. IR (ν cm^{-1}, KBr): 3128 m (N–H), 1620 s (C=O), 1460 s (thioamide I), 1397 s (thioamide II), 952 s (N–N), 792 m (C–S). UV–vis (ν cm^{-1}): 18 020, 24 690, 33 330. MS (FAB): $m/z = 625$ $[M]^+$.

[Fe(Hmtbh)(mtbh)]: orange brown. This complex was prepared by refluxing a solution of H_2mtbh with $[Fe(acac)_3]$ in $CHCl_3$ for 2 h. The resulting precipitate was filtered off, washed successively with $CHCl_3$ and MeOH and dried in vacuo. Anal. Found (%): C, 56.8; H, 4.2; N, 8.3; Fe, 8.4. Calculated (%) for $C_{30}H_{25}FeN_4O_4S_2$ (625.2): C, 56.6; H, 3.9; N, 8.9; Fe, 8.9. IR (ν cm^{-1}, KBr): 3123 m (N–H), 1622 (C=O), 1600 s (NCO), 1462 s (thioamide I), 1393 s (thioamide II), 956 s (N–N), 780 m (C–S). UV–vis (ν cm^{-1}): 19 800, 21 200, 32 400. MS (FAB): $m/z = 625$ $[M]^+$.

3.4. Physical measurement

By following the standard procedures, the complexes were analyzed for their metal content, after decomposition with a mixture of conc. HNO_3 and HCl, followed by conc. H_2SO_4. Chloride was determined as AgCl [46]. Carbon, hydrogen and nitrogen were estimated on a Carlo Erba 1108 model microanalyser. Magnetic susceptibility measurements were made at room temperature on a Cahn Faraday balance using $Hg[Co(NCS)_4]$ as calibrant. Electronic spectra were recorded on a CARY 100 Varian EL01055314 UV–visible spectrophotometer as Nujol mulls [47]. IR spectra were recorded in the 4000–400 cm^{-1} region as KBr pallets on a Jasco FT/IR-5300 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a JEOL AL300 FT NMR spectrometer using TMS as internal reference. ESR spectra were recorded on an X-band spectrometer model EPR-112 using DPPH as a <g> marker. FAB mass spectra were obtained

on a JEOL SX 1021 DA-6000 mass spectrometer using nitrobenzyl alcohol (NBA) as a matrix. The Mössbauer spectrum was recorded at 77 K using a custom made cryostate (Prototech Ltd.) on a Cryophysics MS-1 microprocessor-controlled spectrometer. The source was 25 mCi, 925 MBq ^{57}Co that was mounted on a MVT-1000 transducer. The laser velocity was calibrated with an MVC-450 driving angle waveform generated by an MD4-1200 driver unit and a DFG-1000 digital function generator. Data collection utilized a PC-driven CMCA-550 MCA/PHA card from Wissel GmbH, Germany. The collected data were then fitted to Lorentzian line shapes using Normos V 9.0 program.

3.5. Cell culture

The HT29 colon cancer cell line was obtained from ATCC. Cells were grown in DMEM supplemented with 10% fetal calf serum, 1% antibiotic–antimycotic solution (GIBCO) containing penicillin G sodium, streptomycin sulphate and amphotericin B in humidified air and 5% CO_2 at 37 °C. Cells were harvested after 48 h upon treatment with various concentrations of H_2mtbh, [Mn(Hmtbh)$_2$] or [Fe(Hmtbh)(mtbh)]. Cells were lysed in RIPA buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing 10 mM DTT, 1 mM PMSF and 1% protease inhibitor cocktail (Sigma).

3.6. N-Myristoyltransferase assay

NMT activity was measured as previously described [48]. Briefly, [^{3}H]myristoyl-CoA was synthesized enzymatically according to previously described procedure [48]. The reaction mixture contained 40 mM Tris–HCl, pH 7.4, 0.1 mM EGTA, 10 mM $MgCl_2$, 5 mM ATP, 1 mM LiCoA, 1 μM [^{3}H]myristic acid (7.5 μCi) and 0.3 unit/ml *Pseudomonas* acyl-CoA synthetase in a total volume of 200 μl. The reaction was carried out for 30 min at 30 °C. The conversion to [^{3}H]myristoyl-CoA was generally greater than 95%. The assay mixture contained 40 mM Tris–HCl, pH 7.4, 0.5 mM EGTA, 0.45 mM 2-mercaptoethanol, 1% Triton X-100, pp60^{src} derived peptide (500 μM) and human recombinant NMT (0.2 μg) in a total volume of 25 μl. The transferase reaction was initiated by the addition of freshly generated [^{3}H]myristoyl-CoA and was incubated at 30 °C for 30 min. The reaction was terminated by spotting 15 μl aliquots of incubation mixture onto P81 phosphocellulose paper discs and drying under stream of warm air. The P81 phosphocellulose paper discs were washed in two changes of 40 mM Tris–HCl, pH 7.3 for 60 min. The radioactivity was quantified in 7.5 ml of Beckman Ready Safe Liquid Scintillation mixture in a Beckman Liquid Scintillation Counter. One unit of NMT activity was expressed as 1 pmol of myristoyl peptide formed per minute.

3.7. N-myristoyltransferase inhibition assay

NMT inhibition assay was carried out as described earlier [39]. NMT activity was measured in the presence or absence of ligand or metal complexes. [Mn(Hmtbh)$_2$] and [Fe(Hmtbh)(mtbh)] were assayed for their inhibitory activity against standard human NMT (hNMT). Purified recombinant hNMT (0.2 μg/assay) was assayed [48] in the presence of pp60^{src} derived peptide substrate and metal complexes. A control experiment was performed in the absence of ligand or metal complex and the hNMT activity was considered as 100%. All other conditions were as described above.

3.8. MTT assay

The MTT assay was employed to determine the cytotoxicity of the ligand and the metal complexes. The MTT assay was performed using a standard procedure [49]. HT29 cells were grown in a 96 well plate and treated with various concentrations of H_2mtbh, [Mn(Hmtbh)$_2$] or [Fe(Hmtbh)(mtbh)]. IC_{50} values were calculated by plotting concentration against percentage cell proliferation.

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