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Synthesis, antimicrobial activity and chemotherapeutic potential of inorganic derivatives of 2-(4′-thiazolyl)benzimidazole{thiabendazole}: X-ray crystal structures of $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ and $TBZH_2NO_3$ (TBZH = thiabendazole)

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Abstract

Thiabendazole (TBZH) reacts with iron(III) nitrate causing protonation of the ligand to yield the nitrate salt $[TBZH_2NO_3]$ (**1**). Reaction of TBZH with copper(II) acetate results in the deprotonation of the ligand yielding $[Cu(TBZ)_2 \cdot (H_2O)_2]$ (**2**). Reactions of TBZH with the chloride, nitrate and butanedioate salts of copper(II) yields $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**), $[Cu(TBZH)_2(NO_3)_2]$ (**4**) and $[Cu(TBZH)(O_2C–CH_2CH_2–CO_2)]$ (**5**), respectively. The TBZH acts as a neutral chelating ligand in **3**–**5**. Molecular structures of **1** and **3** were determined crystallographically. In **1**, the asymmetric unit contains one $TBZH_2^+$ cation and one NO_3^- anion. The structure of **3** comprises a five coordinate copper centre with the metal bound to two chelating TBZH ligands and one chloride. The geometry is best described as trigonal bipyramidal. Hydrogen bonding connects the complex cation with the uncoordinated chloride anion and the water and ethanol solvate molecules. Compound **1** and the copper complexes **2**–**5**, the metal free ligands and a number of simple copper(II) salts were each tested for their ability to inhibit the growth of *Candida albicans*. The metal free TBZH and its nitrate salt (**1**) exhibited very poor activity. Complex **2**, in which the TBZH is present as an anionic ligand (TBZ^-), exhibits moderate activity towards the pathogen. Chelation of the neutral TBZH to copper centres (complexes **3**–**5**) results in potent anti-*candida* activity. The dimethyl sulphoxide (DMSO) soluble complexes **3** and **4**, along with metal free TBZH were assessed for their cancer chemotherapeutic potential towards two human epithelial-derived cancer model cell lines. Complexes **3** and **4** displayed similar dose-dependent cytotoxicity in both cell lines with IC_{50} values of approximately 50 μM, which were found to be significantly lower than that for metal free TBZH.

Keywords: Anti-fungal; *Candida*; Chemotherapeutic potential; Metal complexes; Thiabendazole

1. Introduction

Candida albicans is a commensal of the human body and is considered to be an important fungal pathogen. Opportunistic infection can lead to the development of systemic candidosis which is often fatal in

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immunocompromised patients [1–4]. Existing therapies for systemic fungal infections rely on the use of polyene and azole anti-fungal drugs, such as nystatin and ketoconazole. Resistence to these drugs has been reported [5] and this reduces the efficacy of the therapy and can ultimately lead to the death of the patient. Mechanisms that confer antifungal drug resistence in yeast include an increase in the expression of drug efflux pumps which remove the drug from the cell before a toxic concentration can be reached [6], alterations in the target of the drug and variations in the ergosterol biosynthetic pathway [7]. The problems associated with the polyene and azole drugs have resulted in a search for possible alternative anti-fungal agents [8]. Reports have appeared in the literature describing the anti-fungal activity of metal complexes [9]. Due to the possibility of a difference in mode of anti-fungal activity metal-based drugs may represent a novel group of anti-mycotic agents which could have potential applications as pharmaceuticals.

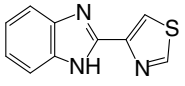
Recently, we have shown that a range of carboxylate and dicarboxylate complexes incorporating transition metal centres and including the *N*,*N*′-donor ligand 1,10-phenanthroline (phen) are potent in vitro inhibitors of the growth of *C. albicans* [10–16].

Furthermore, we have shown that by changing the structural nature of the chelating phenanthroline molecules it is possible to generate complexes that are very active at much lower concentrations [17]. Experiments with 1,7-phenanthroline and 4,7-phenanthroline demonstrated that ligands with chelating ability appeared to be desirable for anti-fungal activity [18]. However, when the 1,10-phenanthroline is replaced by the structurally similar *N*,*N*′-donor ligand 2,2′-bipyridine (bipy) complexes devoid of anti-*candida* activity are obtained [16]. Significantly, when the 1,10-phenanthroline itself is tested against the *candida* it generally exhibits superior activity to that of the metal complexes and activity comparable to the prescription drugs. Other workers have recently reported very good anti-*candida* activity for several new organic 1,10-phenanthroline derivatives [19]. We believe that the so-called "metal free" 1,10-phenanthroline is probably coordinating to metal ions that are present in trace amounts in the growth medium and that it is these resulting metal–phenanthroline complexes that are responsible for the high anti-*candida* activity.

The mode of action of 1,10-phenanthroline and a number of our potent anti-*candida* metal complexes {M = Cu(II), Mn(II) or Ag(I)} [20] was examined. The phen and its metal complexes had minimum inhibitory concentrations (MICs) in the range 1.25–5 μg/ml and at concentration of 10 μg/ml they displayed some fungicidal activity.

Yeast cells exposed to these drugs showed a diminished ability to reduce 2,3,5-triphenyltetrazolium chloride (TTC), indicating a reduction in respiratory function. Treating exponential and stationary phase yeast cells with phen and the Cu(II) and Mn(II) complexes caused a dramatic increase in oxygen consumption. All of the drugs promoted reduction in levels of cytochrome *b* and *c* in the cells, whilst the Ag(I) complex also lowered the level of cytochrome *aa*. Cells treated with phen and the Cu(II) and Ag(I) species showed reduced levels of ergosterol whilst the Mn(II) complex induced an increase in the sterol concentration. The general conclusion was that phen and its Cu(II), Mn(II) and Ag(I) complexes damage mitochondrial function and uncouple respiration. The fact that these drugs were not uniformly active suggests that their biological activity has a degree of metal-ion dependency. The effect of these drugs on the structure of yeast and mammalian cell organelles and the integrity of cellular DNA was also studied [21]. The conclusion was that phen and the metal–phen complexes have the potential to induce apoptosis in fungal and mammalian cells. 1,10-Phenanthroline and its metal complexes represent a novel set of highly active anti-fungal agents whose mode of action is significantly different to that of the state-of-the-art polyene and azole prescription drugs. In an effort to extend this class of novel drug, we have been studying metal complexes containing benzimidazole-based ligands. Benzimidazole and many of its derivatives exhibit a variety of biological actions, including antibacterial, antiviral, anticancer and anti-fungal activity [22].

2-(4′-Thiazolyl)benzimidazole{thiabendazole (TBZH)} (Fig. 1) is a well-known anthelmintic which is non-toxic to humans [23] and it also has applications as a fungicide in agriculture [24]. Because of its structural similarity to the chelating agents 2,2′-bipyridine and 1,10-phenanthroline, we were prompted to try and generate metal complexes of it. Further interest is derived from TBZH as it can act as both an acid and a base making it possible to generate inorganic compounds in which it can be either neutral, anionic or cationic. Reports of the biological activity of metal complexes of this potential *N*,*N*′-donor chelating ligand are quite rare. In the present paper, we report the synthesis, characterisation and the fungitoxic activity of inorganic derivatives of TBZH. To date TBZH is the first *N*,*N*′-donor ligand we have studied that, in vitro, exhibits poor anti-*candida* activity on its own but when complexed to a copper(II) centre becomes a relatively potent drug. In light of the fact that



Thiabendazole

Fig. 1. The structure of thiabendazole.

very little is known about the biological properties of metal complexes of TBZH, we also investigated the chemotherapeutic potential of the free ligand and two of the novel complexes towards two tumourigenic human model cell lines.

2. Experimental

2.1. Chemistry

Chemicals were purchased from commercial sources and used without further purification. IR spectra were recorded in the region 4000–400 cm^{-1} on a Nicolet-400 Impact spectrometer. Magnetic susceptibility measurements were made using a Johnson Matthey Magnetic Susceptibility balance. $[HgCo(SCN)_4]$ was used as a reference. Satisfactory microanalytical data for the complexes were reported by the Microanalytical Laboratory, University College Cork, Ireland. $[Cu(O_2C–CH_2CH_2–CO_2)]\{HO_2C–CH_2CH_2–CO_2H = \text{butanedioic acid}\}$ was synthesised using a method previously published [12].

2.1.1. *[TBZH$_2$NO$_3$] (**1**)*

To a solution of $Fe(NO_3)_3 \cdot 9H_2O$ (0.5 g, 1.24×10^{-3} mmol) in ethanol (100 cm^3) was added thiabendazole (TBZH) (0.5 g, 2.47×10^{-3} mmol) and the resulting dark red solution was refluxed for 3 h. Upon standing colourless crystals of the product formed which were filtered and air-dried. Yield: 0.1478 g (45.3%). Calc: C, 45.62; H, 2.68; N, 21.28. Found: C, 45.45; H, 3.02; N, 21.18%. IR (KBr): 3408, 3084, 1635, 1586, 1385, 1347, 1304, 1043, 825, 739, 535 cm^{-1}. Solubility: soluble in ethanol and methanol.

2.1.2. *[Cu(TBZH)$_2$ · (H$_2$O)$_2$] (**2**)*

To a solution of thiabendazole (TBZH) (2.01 g, 10.00 mmol) in ethanol (100 cm^3) was added $[Cu_2(CH_3CO_2)_2 \cdot 2H_2O]$ (1.00 g, 5.00 mmol) and the resulting dark green solution was refluxed for 4 h. The dark green solid which deposited was filtered off, washed with water and ethanol, and then air-dried.Yield: 2.40 g (96%). Calc: C, 48.03; H, 3.22; N, 16.81. Found: C, 48.99; H, 2.60; N, 16.74%. IR (KBr): 3427, 3082, 1605, 1474, 1409, 1359, 1294, 1269, 1228, 1015, 933, 908, 875, 834, 744 cm^{-1}. μ_{eff}: 1.64 B.M. Solubility: insoluble in water, ethanol, methanol, acetone and trichloromethane.

2.1.3. *[Cu(TBZH)$_2$Cl]Cl · H$_2$O · EtOH (**3**)*

To a solution of thiabendazole (TBZH) (3.14 g, 7.40 mmol) in ethanol (100 cm^3) was added $CuCl_2 \cdot 2H_2O$ (1 g, 7.40 mmol) and the resulting light green solution was refluxed for 4 h. The light green solid which deposited was filtered off, washed with water and ethanol, and then air-dried. Yield: 2.70 g (64%). Calc: C, 43.12; H, 3.17; N, 13.98. Found: C, 43.12; H, 3.28; N, 13.91%. IR (KBr): 3443, 3328, 3221, 3074, 2967, 2828, 2764, 1630, 1597, 1513, 1491, 1466, 1441, 1326, 1294, 1228, 1189, 1080, 1048, 1015, 998, 933, 875, 842, 744, 670 cm^{-1}. μ_{eff}: 1.57 B.M. Solubility: insoluble in water, ethanol, methanol, acetone and trichloromethane.

2.1.4. *[Cu(TBZH)$_2$(NO$_3$)$_2$] (**4**)*

To a solution of thiabendazole (TBZH) (1.80 g, 9.02 mmol) in ethanol (100 cm^3) was added $Cu(NO_3)_2 \cdot 2H_2O$ (1.00 g, 4.29 mmol) and the resulting lime green solution was refluxed for 4 h. The lime green solid which deposited was filtered off, washed with water and ethanol, and then air-dried. Yield: 2.57 g (98%). Calc: C, 40.68; H, 2.37; N, 18.48. Found: C, 40.54; H, 2.39; N, 18.51%. IR (KBr): 3098, 2360, 1593, 1517, 1441, 1409, 1384, 1329, 1289, 1228, 1015, 998, 933, 875, 842, 769, 744 cm^{-1}. μ_{eff}: 1.71 B.M. Solubility: insoluble in water, ethanol, methanol, acetone and trichloromethane.

2.1.5. *[Cu(TBZH)(bda)] (**5**)*

To a solution of thiabendazole (TBZH) (0.60 g, 3.00 mmol) in ethanol (50 cm^3) was added $[Cu(O_2C–CH_2CH_2–CO_2)]$ (0.50 g, 1.53 mmol) and the blue suspension was refluxed for 4 h. The resulting blue solid which deposited was filtered off, washed with water and ethanol, and then air-dried. Yield: 0.62 g (69%). Calc: C, 44.15; H, 2.91; N, 11.03. Found: C, 44.38; H, 2.85; N, 11.67%. IR (KBr): 3427, 3115, 2918, 2352, 1557, 1425,1409, 1285, 1228, 1187, 1015, 974, 941, 875, 834, 777, 637 cm^{-1}. μ_{eff}: 1.74 B.M. Solubility: insoluble in water, ethanol, methanol, acetone and trichloromethane.

2.2. X-ray crystallography

The two data sets were collected at 150°(2) K on a Bruker SMART 1000 diffractometer using Mo Kα radiation ($\lambda = 0.71073$ Å). Each was solved by direct methods and refined by full-matrix least-squares on F^2. All the non-hydrogen atoms were refined with anisotropic atomic displacement parameters and hydrogen atoms bonded to carbon were inserted at calculated positions using a riding model. In **1**, the hydrogen atoms bonded to nitrogen were located in the difference map but then inserted at calculated positions using a riding model. In **3**, the hydrogen atoms bonded to nitrogen were located from difference maps and not further refined; that bonded to the ethanol oxygen atom in **3** was treated in the same way. Hydrogen atoms bonded to water molecules were not included in the models. Details of the collection and refinement are given in Table 1 . All programmes used in the structure solution and refinement are contained in the SHELXTL package [25].

Table 1
Crystal data and structure refinement for **1** and **3**

Compound	$[TBZH_2NO_3]$ (**1**)	$[Cu(TBZ)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**)
Empirical formula	$C_{10}H_8N_4O_3S$	$C_{22}H_{22}Cl_2CuN_6O_2S_2$
Formula weight	264.26	601.02
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2/c$
a (Å)	4.8074(7)	16.2813(8)
b (Å)	13.812(2)	11.3482(6)
c (Å)	16.619(3)	15.6061(8)
β (°)	97.779(2)	118.075(1)
Volume (Å^3)	1093.4(3)	2544.1(2)
Z	4	4
Density (calc) (Mg/m^3)	1.605	1.569
Absorption coefficient (mm^{-1})	0.303	1.266
$F(000)$	544	1228
Crystal size (mm^3)	$0.42 \times 0.17 \times 0.09$	$0.20 \times 0.15 \times 0.15$
Crystal description	Yellow block	Green needle
θ range (°)	1.92–25.00	1.42–28.92
Index ranges	$-5 \leqslant h \leqslant 5$, $-16 \leqslant k \leqslant 15$, $-19 \leqslant l \leqslant 19$	$-21 \leqslant h \leqslant 22$, $-15 \leqslant k \leqslant 14$, $-20 \leqslant l \leqslant 20$
Reflections collected	7359	29,636
Independent reflections [R_{int}]	1926 [0.0275]	6164 [0.0484]
T_{max}, T_{min}	1.00000, 0.771000	0.928078, 0.762571
Data/restraints/parameters	1926/0/163	6164/0/316
Goodness-of-fit on F^2	1.060	1.013
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0376$, $wR_2 = 0.0987$	$R_1 = 0.0484$, $wR_2 = 0.1321$
R indices (all data)	$R_1 = 0.0434$, $wR_2 = 0.1035$	$R_1 = 0.0820$, $wR_2 = 0.1534$
Largest difference peak and hole (e Å^{-3})	0.481 and −0.537	0.820 and −0.561

2.3. Anti-candida testing

Candida albicans isolate was obtained commercially from Oxid Culti-loops (ATCC 10241). The isolate was stored on Sabouraud dextrose agar (SDA) plates at 4 °C. Culture conditions and measurement of drug minimum inhibitory concentrations (MICs) were as previously described [11].

2.4. Cytotoxicity testing

Dimethyl sulphoxide (DMSO) and all cell culture reagents and media were purchased from Signa–Aldrich Ireland, Ltd, unless otherwise stated.

2.5. Cell lines and cell culture

Cytotoxicity assays were performed using two human model cell lines in order to assess the cancer chemotherapeutic potential of metal free TBZH and complexes **3** and **4** (the other complexes were not soluble in DMSO). The human malignant melanoma (melanocyte) skin cell line (SK-MEL-31) and squamous carcinoma tongue cell line (CAL-27) were purchased from the American Type Culture Collection, Manassas. SK-MEL-31 cells were grown as a monolayer in Eagle's minimum essential medium, supplemented with 2 mM L-glutamine and Earle's balanced salt solution, containing 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin supplemented to contain 15% (v/v) foetal bovine serum (Flow laboratories, Herts, UK). The CAL-27 cells were grown in Dulbecco's modified Eagle's medium, supplemented with 4 mM L-glutamine, containing 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin supplemented to contain 15% (v/v) foetal bovine serum. Both model cells were grown at 37 °C in a humidified atmosphere, in the presence of 5% CO_2 and were in the exponential phase of growth at the time of assay.

2.6. Assessment of cytotoxicity, using MTT assay

Test compounds were dissolved in DMSO, diluted in culture media and used to treat the two model cells over a drug concentration range 0.1–1000 μM for a period of 96 h. SK-MEL-31 and CAL-27 cells were seed at a density of 3.5×10^4 and 5×10^3 cells/well, respectively, into sterile 96 well flat-bottomed plates (Falcon Plastics, Decton Dickinson) and grown in 5% CO_2 at 37 °C. A miniaturised viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was carried out according to the method described by Mosman [26]. The IC_{50} value was calculated for each drug and used as a means for comparing the toxicity of each of the derivatives tested. Consequently, IC_{50} was

defined as the drug concentration causing a 50% reduction in cellular viability. Each assay was carried out using five replicates and repeated on at least three separate occasions. Viability was calculated as a percentage of solvent treated control cells, and expressed as percentage of control. The significance of any reduction in cellular viability was determined using one-way ANOVA (analysis of variance). A probability of 0.05 or less was deemed statistically significant.

3. Results and discussion

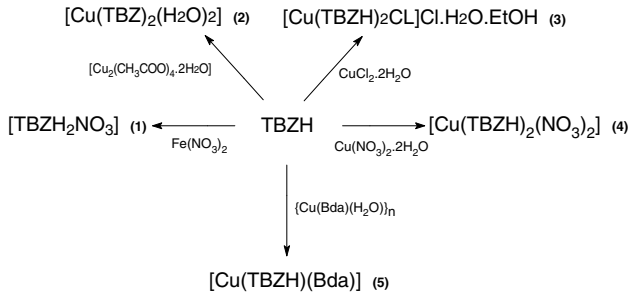
3.1. Synthesis and characterisation of the compounds

Five inorganic derivatives of TBZH in which the ligand is protonated {$[TBZH_2NO_3]$ (**1**)}, deprotonated {$[Cu(TBZ)_2 \cdot (H_2O)_2]$ (**2**)} and neutral {$[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**), $[Cu(TBZH)_2(NO_3)_2]$ (**4**) and [Cu(TBZH)(bda)] (**5**)} were generated in moderate to good yield using the reactions shown in Scheme 1. The non-metal-based nitrate salt $[TBZH_2NO_3]$ (**1**) was the major product of a reaction between TBZH and $Fe(III)(NO_3)_3$. Attempts to generate this salt using a direct method involving nitric acid and TBZH have so far been unsuccessful and the mechanism of the formation of the salt is unknown. Hydrated Fe(III) salts are known to act as aqua acids, a fact which could explain the protonation of the TBZH molecule during this reaction. Failure to isolate any iron complex formed during the reaction may well be due to the fact that Fe(III) compounds are not particularly stable.

The formulation of the compounds was assigned on the basis of their elemental analysis, IR spectra and X-ray analysis (for **1** and **3**). In the respective IR spectra the majority of the ligand absorption bands, some of them with changed intensity, appear again in the compounds. In **1**, the N–H stretching band at 3093 cm^{-1} has shifted to 3084 cm^{-1} and has become more intense. Furthermore, the band associated to the imidazolic ν(C=N) at 1577 cm^{-1} in the free ligand has completely disappeared in the cationic $TBZH_2^+$ due to protonation on the benzimidazole imine nitrogen, resulting in delocalisation of the double bond over the N–C–N section. A strong absorption band at approximately 1385 cm^{-1} in the spectrum of **1** (which does not appear for the ligand) is characteristic of a nitrate group [27].

The anionic nature of the ligand in **2** is evident when its spectrum is compared to that of TBZH. The prominent bands at 3093 cm^{-1} (N–H stretching) and at 1093 cm^{-1} (N–H vibration) for the free ligand were absent in the spectrum of **2**. Also for **2**, the band associated to the imidazolic ν(C=N) at 1577 cm^{-1} in the free ligand has shifted to 1605 cm^{-1} due to deprotonation of the imidazolic nitrogen. In the spectra of complexes **3** and **4** the $\nu(C{=}N)_{imidazolic}$ and $\nu(C{=}N)_{thiazolic}$ bands (1577 and 1480 cm^{-1}, respectively) are shifted (to 1597 and 1513 cm^{-1} for **3**; 1593 and 1517 cm^{-1} for **4**) indicating that the ligand is coordinated through the imidazolic and the thiazolic nitrogens. The asymmetrical carboxylate stretching band precludes a similar assignment in the spectrum of **5**. For all four complexes **2**–**5** the C–S stretching band (at 1228 cm^{-1} for the free ligand) remains essentially unchanged suggesting that the sulphur atom in the thiazole ring is uncoordinated. New bands in the spectrum of **4** at approximately 1441 and 1289 cm^{-1} are assigned to an uncoordinated nitrate group whereas a new band at 1329 cm^{-1} is indicative of the presence of a coordinated nitrate. As well as the bands that have been assigned to chelating TBZH ligands the spectrum of **5** has bands that are characteristic of carboxylate anions {$\nu(OCO)_{assym}$ at 1557 cm^{-1} and $\nu(OCO)_{sym}$ at 1409}. The calculated Δ(OCO) value {$\nu(OCO)_{assym} - \nu(OCO)_{sym}$} of 148 cm^{-1} is typical for a carboxylate group bound to a metal in a chelating coordination mode [28]. Whereas the room temperature magnetic susceptibility values for **4** and **5** are close to those expected for simple copper(II) species (i.e., those lacking Cu–Cu interactions) the values for **2** and **3** are slightly lower and some form of antiferromagnetic interaction may be taking place in these complexes [29]. With the exception of the nitrate salt all of the complexes are effectively insoluble in common solvents. Complexes **3** and **4** were found to be soluble in DMSO.

Crystals suitable for X-ray analysis were isolated for compounds **1** and **3**. The structure of $[TBZH_2NO_3]$ (**1**) is shown in Figs. 2 and 3, and bond lengths and angles are



Scheme 1.

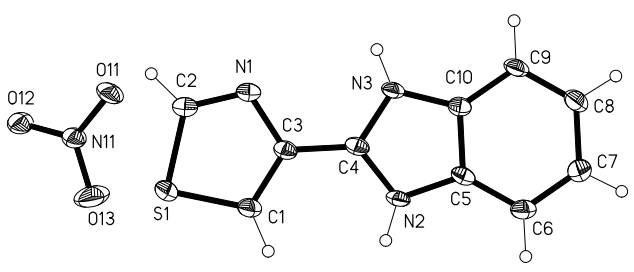


Fig. 2. The asymmetric unit of $[TBZH_2NO_3]$ (**1**).

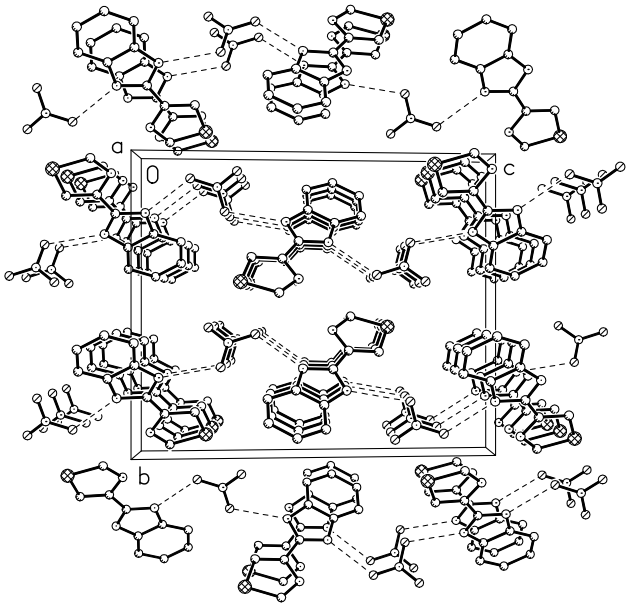


Fig. 3. A view of the π–π stacking in $[TBZH_2NO_3]$ (**1**).

listed in Table 2. The asymmetric unit contains one protonated $TBZH_2$ cation and one nitrate anion. The TBZH is protonated on the benzimidazole imine nitrogen, resulting in delocalisation of the double bond over the N–C–N section (Table 2). The cations are hydrogen bonded (Table 3) to nitrate anions via each of the NH groups, resulting in a chain along which adjacent $TBZH_2$ units are oriented at approximately right angles to each other. There is significant ππ-stacking between adjacent $TBZH_2$ cations with an interplanar distance ca. 3.3 Å.

The structure for $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**) is shown in Figs. 4–6 and selected bond lengths and angles are listed in Table 4. The copper ion is five-coordinate, bound to two TBZH ligands via α-diimine units and to one chloride anion (Fig. 4). The geometry at copper is irregular but perhaps best described as trigonal bipyramidal with the benzimidazole nitrogen atoms N2 and N12 as apical donors (N2–Cu–N12 171.9(1)°). The benzimidazole NH groups N1 and N11 are hydrogen bonded to Cl1 of a neighbouring cation and to Cl2, respectively (Table 5 and Fig. 5). The OH protons of the solvate water and ethanol molecules are also involved on hydrogen bonding (Fig. 4), resulting in a 3D network of hydrogen bonding extending through the lattice and supported by π–π-interactions (Fig. 6).

3.2. Biological activities

The nitrate salt of TBZH (**1**), all of the copper complexes {**2**–**5** and $[Cu(O_2C–CH_2CH_2–CO_2)]$}, the free ligands and a selection of simple copper salts were each tested for their ability to inhibit the growth of *C. albicans*

Table 2
Bond lengths (Å) and angles (°) for $[TBZH_2NO_3]$ (**1**)

C(1)–C(3)	1.364(3)
C(1)–S(1)	1.698(2)
S(1)–C(2)	1.7209(19)
C(2)–N(1)	1.303(3)
N(1)–C(3)	1.379(2)
C(3)–C(4)	1.441(3)
C(4)–N(2)	1.338(2)
C(4)–N(3)	1.341(2)
N(2)–C(5)	1.385(3)
C(5)–C(6)	1.390(3)
C(5)–C(10)	1.398(3)
C(6)–C(7)	1.379(3)
C(7)–C(8)	1.407(3)
C(8)–C(9)	1.378(3)
C(9)–C(10)	1.389(3)
C(10)–N(3)	1.381(3)
N(11)–O(13)	1.235(2)
N(11)–O(11)	1.254(2)
N(11)–O(12)	1.258(2)
C(3)–C(1)–S(1)	110.24(15)
C(1)–S(1)–C(2)	89.13(10)
N(1)–C(2)–S(1)	115.73(15)
C(2)–N(1)–C(3)	109.33(16)
C(1)–C(3)–N(1)	115.57(18)
C(1)–C(3)–C(4)	124.90(18)
N(1)–C(3)–C(4)	119.53(16)
N(2)–C(4)–N(3)	109.24(17)
N(2)–C(4)–C(3)	125.10(17)
N(3)–C(4)–C(3)	125.66(17)
C(4)–N(2)–C(5)	108.96(15)
N(2)–C(5)–C(6)	131.75(17)
N(2)–C(5)–C(10)	106.31(17)
C(6)–C(5)–C(10)	121.94(18)
C(7)–C(6)–C(5)	116.20(18)
C(6)–C(7)–C(8)	121.90(19)
C(9)–C(8)–C(7)	121.87(19)
C(8)–C(9)–C(10)	116.41(18)
N(3)–C(10)–C(9)	131.64(17)
N(3)–C(10)–C(5)	106.68(16)
C(9)–C(10)–C(5)	121.68(18)
C(4)–N(3)–C(10)	108.80(16)
O(13)–N(11)–O(11)	120.53(17)
O(13)–N(11)–O(12)	119.37(16)
O(11)–N(11)–O(12)	120.09(16)

Table 3
Hydrogen bonds for $[TBZH_2NO_3]$ (**1**) (Å and °)

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠(DHA)
N(2)–H(2A)···O(12)#1	0.88	1.91	2.785(2)	175.3
N(2)–H(2A)···O(13)#1	0.88	2.58	3.213(2)	129.7
N(3)–H(3)···O(11)#2	0.88	1.98	2.856(2)	170.9

Symmetry transformations used to generate equivalent atoms: #1 $-x+1/2, y+1/2, -z+3/2$ and #2 $-x, -y+1, -z+1$.

(Table 6). Butanedioic acid ($HO_2C–CH_2CH_2–CO_2H$) and the simple copper salts are essentially inactive against the pathogen. Furthermore, coordination of butanedioic acid to a copper center {$[Cu(O_2C–CH_2CH_2–CO_2)]$} does not result in any significant improvement in the activity

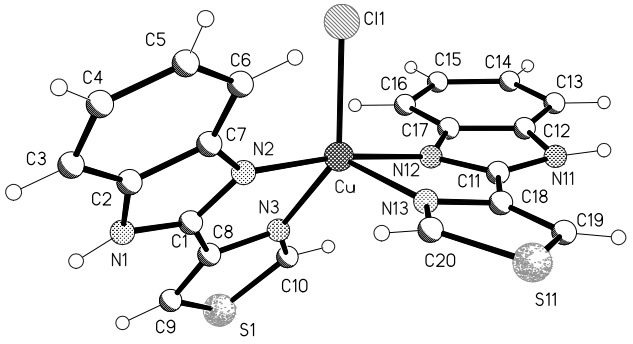


Fig. 4. The structure of the cation in $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**).

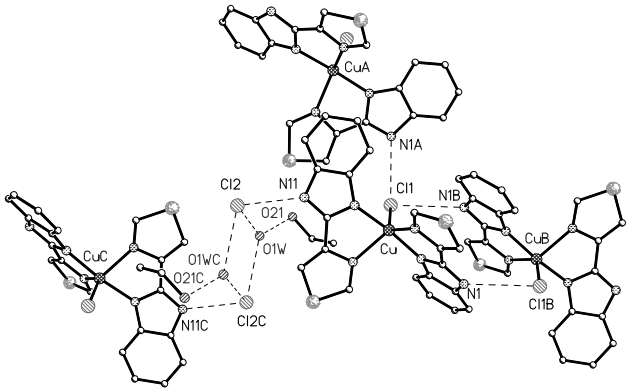


Fig. 5. Hydrogen bonding in $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**).

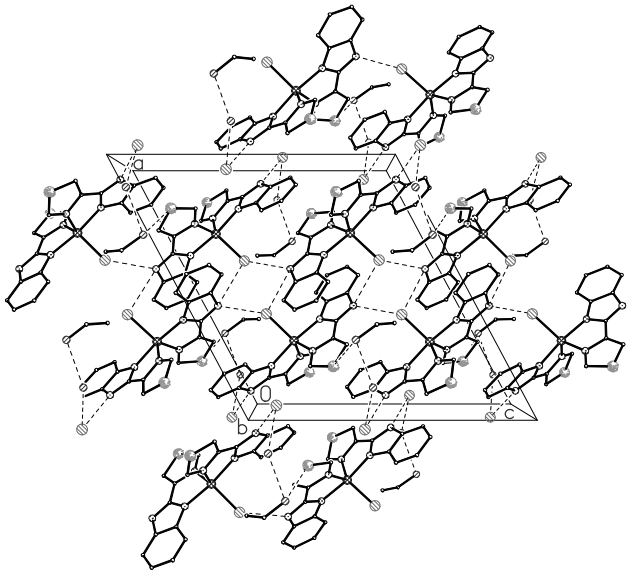


Fig. 6. The packing diagram in $[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**).

Table 4
Selected bond lengths (Å) and angles (°) for $[Cu(TBZ)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**)

Cu–N(2)	1.972(3)
Cu–N(12)	1.975(3)
Cu–N(3)	2.076(3)
Cu–N(13)	2.153(3)
Cu–Cl(1)	2.3050(11)
N(2)–Cu–N(12)	171.91(13)
N(2)–Cu–N(3)	80.75(12)
N(12)–Cu–N(3)	95.98(12)
N(2)–Cu–N(13)	95.27(11)
N(12)–Cu–N(13)	79.98(11)
N(3)–Cu–N(13)	120.82(12)
N(2)–Cu–Cl(1)	95.22(9)
N(12)–Cu–Cl(1)	92.34(10)
N(3)–Cu–Cl(1)	134.35(9)
N(13)–Cu–Cl(1)	104.82(9)

Table 5
Hydrogen bonds for $[Cu(TBZ)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**) (Å and °)

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠(DHA)
N(1)–H(1)···Cl(1)#1	0.91	2.37	3.209(3)	152.2
N(11)–H(11)···Cl(2)#2	0.88	2.18	3.050(3)	168.3
O(21)–H(21)···O(1W)	0.88	1.89	2.732(7)	161.4
N(1)–H(1)···Cl(1)#5	0.91	2.77	3.398(3)	126.6
O1W···Cl2#3			3.187(5)	
O1W···Cl2#4			3.158(4)	

Symmetry transformations used to generate equivalent atoms: #1 $x, -y+1, z+1/2$, #2 $-x, -y, -z$, #3 $x, -y, z+1/2$, #4 $-x, y, -z+1/2$ and #5 $-x+1, -y+1, -z+1$.

Table 6
Anti-*candida* activity[a]

Test compound	% Cell growth
Control	100
Ketoconazole	20
1,10-Phenanthroline	15
$CuCl_2$	95
$CuNO_3$	98
Cu(OAc)2	94
$HO_2C–CH_2CH_2–CO_2H$	98
$[Cu(O_2C–CH_2CH_2–CO_2)]$	95
TBZH	76
$[TBZH_2NO_3]$ (**1**)	60
$[Cu(TBZ)_2(H_2O)_2]$ (**2**)	54
$[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**)	29
$[Cu(NO_3)_2(TBZH)_2]$ (**4**)	30
$[Cu(TBZH)(O_2C–CH_2CH_2–CO_2)]$ (**5**)	19

[a] The compounds were tested at concentrations of 10 μg/ml of aqueous RPMI medium. Complexes **2**–**5** were insoluble in water and were used as suspensions. Yeast cells were grown for 24 h at 37 °C. Results are presented as % cell growth and the effectiveness of the compounds are compared to the growth of the control (no drug added).

of the dicarboxylic acid. The metal free neutral TBZH is a very poor inhibitor of the growth of the pathogen. Compound **1** and complex **2** (in which the ligand is found in its cationic and anionic states, respectively) are both moderate anti-*candida* agents. Significantly when the neutral TBZH is coordinated to a copper center

(complexes **3**–**5**) very potent anti-*candida* drugs are produced. Complex **5** exhibits the greatest fungitoxic activity and indeed is comparable to the prescription drug ketoconazole at this concentration. Preliminary studies on the mode of action of the copper TBZH complexes have revealed that they cause a reduction in the ergosterol content of the fungal cells [30] which was also found to be the case for the phenanthroline complexes previously reported [20].

The chemotherapeutic potential of TBZH and the DMSO soluble complexes **3** and **4** was determined by calculation of IC_{50}. Calculation of this value allows a direct comparison of the cytotoxicity of each of the test agents. The IC_{50} values were calculated using the data presented in Figs. 7 and 8. The values were obtained for each compound and in each cell line (Table 7). TBZH was capable of killing both cancer-derived cell lines only at higher concentrations with IC_{50} value of 453 and 677 μM (equivalent to 91.7 and 136.9 μg/ml), for the tongue and skin cell line, respectively. In the case of compounds **3** and **4**, the IC_{50} values were very similar across the two human model cell lines. They had almost identical IC_{50} values of 55 and 54 μM (equivalent to 33.1 and 31.9 μg/ml), respectively, in the CAL-27 cell line and 50 and 47 μM (equivalent to 39.1 and 30.7 μg/ml), respectively in the SK-MEL-31 cell line. Although the activities of **3** and **4** do not fall within the accepted activity parameters adopted for in vitro screening (i.e., IC_{50} values not exceeding 4 μg/ml) [31] the results suggest the chemotherapeutic potential of TBZH is significantly enhanced upon coordination to a metal centre. Furthermore, the cytotoxic activity of all three compounds is concentration dependent for both cell lines (Figs. 7 and 8).

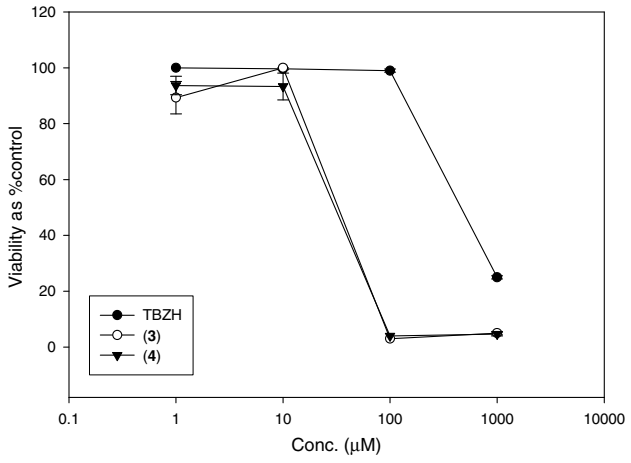


Fig. 7. Effects of TBZH, **3** and **4** on the viability of CAL-27 cells following continuous incubation with increasing drug concentration (0.1–1000 μM) for 96 h. Bars indicate standard error of the mean (SEM) and results were statistically significant from control at $p < 0.05$.

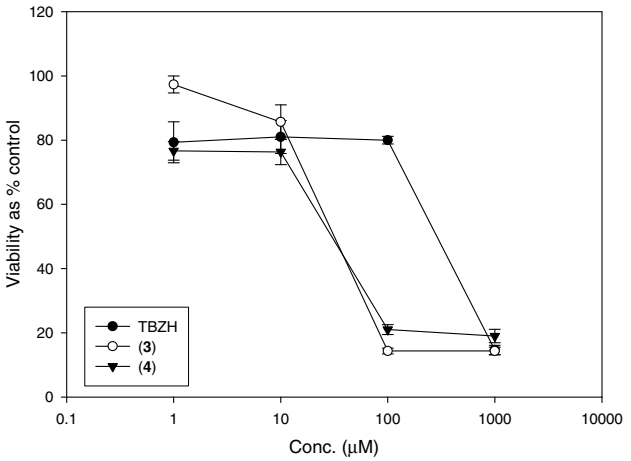


Fig. 8. Effects of TBZH, **3** and **4** on the viability of SK-MEL-31 cells following continuous incubation with increasing drug concentration (0.1–1000 μM) for 96 h. Bars indicate standard error of the mean (SEM) and results were statistically significant from control at $p < 0.05$.

Table 7
The chemotherapeutic potential of TBZH **3** and **4**

Test compound	Toxicities (IC_{50}, μM)	
	CAL-27 (means ± SD)	SK-MEL-31 (means ± SD)
TBZH	676.7 ± 12.0	453.3 ± 66.0
$[Cu(TBZH)_2Cl]Cl \cdot H_2O \cdot EtOH$ (**3**)	55.0 ± 0.0	49.5 ± 7.7
$[Cu(NO_3)_2(TBZH)_2]$ (**4**)	54.0 ± 2.5	46.7 ± 5.0

Cancer chemotherapeutic potential of complexes **3** and **4** along with metal free TBZH in CAL-27 and SK-MEL-31, following continuous incubation for 96 h in the concentration range 0.1–1000 μM, using MTT assay. A graph of viability as % of solvent treated control verses drug concentration was used to calculate IC_{50} values (μM), (means ± SD; $n = 5$).

4. Conclusion

Neutral thiabendazole (TBZH) when uncoordinated to a metal centre is a poor anti-*Candida* agent and has very little chemotherapeutic potential. Protonation of TBZH to form the nitrate salt **1** and its deprotonation to yield the complex **2** results in only moderate improvement in its anti-*candida* activity. Complexes **3**–**5**, in which the TBZH is present as a neutral chelating ligand, are all potent anti-*candida* agents with five possessing activity comparable to the prescription drug ketoconazole. Coordination of neutral TBZH to a copper centre in complexes **3** and **4** resulted in a significant increase in its chemotherapeutic potential.

5. Supplementary data

Crystallographic data have been deposited with the CCDC (12 Union Road, Cambridge, CB2 1EZ, UK)

and are available on request quoting the deposition numbers 219675 and 219676, respectively.

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