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Synthesis and antimicrobial activity of copper(II) and manganese(II) α,ω-dicarboxylate complexes

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Abstract

Copper(II) α,ω-dicarboxylate complexes of general formulae, $[Cu(O_2C(CH_2)_nCO_2)]\cdot xH_2O$, $[Cu(O_2C(CH_2)_nCO_2)(phen)_2]\cdot xH_2O$ and $[Cu(O_2C(CH_2)_nCO_2)(bipy)_y]\cdot xH_2O$ ($n = 1$–8; $y = 1$, 2; phen = 1,10-phenanthroline; bipy = 2,2′-bipyridine) were synthesised. These copper complexes, some related manganese(II) complexes and the metal-free ligands were screened *in vitro* for their ability to inhibit the growth of *Candida albicans*. Metal-free 1,10-phenanthroline and all of the copper(II) and manganese(II) phenanthroline complexes were potent growth inhibitors, with only one bipyridine complex, $[Cu(O_2C(CH_2)CO_2)(bipy)_2]\cdot 2H_2O$, having moderate activity. The remaining substances were effectively inactive. Complexes which were active against *C. albicans* also proved effective against *C. glabrata, C. tropicalis* and *C. kreusi* with the manganese complexes retaining superior activity. For the phenanthroline complexes the active drug species is thought to be the dication $[M(phen)_2(H_2O)_n]^{2+}$ (M = Cu, Mn). *Escherichia coli* and *Staphylococcus aureus* were resistant to all of the metal complexes and also to metal-free 1,10-phenanthroline. Only the copper phenanthroline complexes showed intermediate activity against *Pseudomonas aeruginosa*.

Introduction

Burn wound infections are a major source of morbidity and mortality in burn patients. The injury disrupts both the normal skin barrier and many of the systemic host defense mechanisms that prevent infection. When skin is burned, it is susceptible to colonization by microbial pathogens including *Staphylococcus aureus, Escherichia coli* and *Klebsiella* species (Revathi *et al.* 1998). In addition, a synergistic relationship can exist between the yeast *Candida albicans* and *Pseudomonas aeruginosa* in burn patients (Neely *et al.* 1986). The yeast *Candida albicans* is an important fungal pathogen especially in immunocompromised individuals. As a result of an increase in the use of anti-*Candida* agents, the incidence of resistance of the yeast, particularly after long term suppressive therapy, has increased dramatically. In addition, a rapid increase in infection caused by other species of *Candida*, such as *C. krusei, C. tropicalis* and *C. glabrata* has arisen (Georgopapadakou & Walsh 1996).

A number of reports have appeared in the literature highlighting the use of transition metal complexes as both antibacterial and antifungal agents. For example, the copper(II), cobalt(II) and nickel(II) complexes of Schiff base ligands derived from 2-substituted anilines and salicylaldehyde exhibit good broad-spectrum antifungal and antibacterial activity *in vitro* (Parashar *et al.* 1988). The family of Schiff base complexes of general formula (*N*-salicylidene-L-glutamato)(Q)copper(II) (Q = a quinoline-type ligand) showed good activities against *S. aures* but were only moderately active against *E. coli* and *C. albicans* (Valent *et al.* 1993). Diacetato copper(II), cobalt(II) and nickel(II) complexes incorporating a neutral heterocyclic hydrazone ligand, prepared by

condensing 2-acetylfuran with either iminodiacetic acid dihydrazide or 2,6-dicarboxylic acid dihydrazide, showed good *in vitro* antimicrobial activities against the bacteria *S. aureus* and *E. coli* and the fungi *Aspergillus niger* and *C. albicans* (Sharma *et al.* 1992). Divalent metal complexes of general formula $[ML_2]$ (M = Mn(II), Co(II), Ni(II), Cu(II), Zn(II); L = monoanionic 2-substituted phenylurea $H_2NCONHC_6H_4R$ (R = OH, SH, CO_2H)) displayed good activities against *S. aureus*, *E. coli*, *A. niger* and *C. albicans* (Sharma & Parashar 1988). DMSO solutions of the binuclear copper(II) complexes $[Cu_2(ROCH_2CO_2)_4(apy)_2]$ ($ROCH_2CO_2$ = aryloxyacetato; apy = antipyrene) have good antimicrobial activity, being most efficient against *C. albicans* and *Bacillus subtilis* (Plesch *et al.* 1987). Whereas copper(II) sulphate alone did not have any antimicrobial activity an *in vivo* study conducted on chickens showed that addition of a combination of a simple water soluble copper(II) salt (e.g. $CuSO_4{\cdot}5H_2O$) and ammonium 2-methylpropionate to the poultry feed inhibited the growth and proliferation of *C. albicans*, *E. coli* and *Salmonella* (Das 1973). Similar trends were observed when the same salt mixture was employed *in vitro*. It has been claimed that monocopper(II) citrate is an effective antimicrobial agent and that, furthermore, the complex suppresses the biodeterioration of metalworking fluid (Maurer & Shringapurey 1977, 1978). Subsequent studies revealed that the complex temporarily inhibits the growth of *P. aeruginosa* and *C. tropicalis* in laboratory media (Piet & Rossmoore 1985). Copper(II) complexes of the type [Cu(L)X] (L = tridentate anion of 2-acetylpyridine-*N*-diethylthiosemicarbazone; X = Cl or Br) possess broad-spectrum antifungal activity *in vitro*, and the greater growth inhibition by the bromo complex was explained on the basis of its lower Cu(II)/Cu(I) redox potential (Kumbhar *et al.* 1991). Solutions of copper(II), cobalt(II) and nickel(II) complexes of 1-thiocarbamyl-3,5-dimethylpyrazole in DMSO are quite active *in vitro* against a range of bacteria but are ineffective against *C. albicans* (Chatterjee *et al.* 1986). Detailed investigations with the copper complex revealed that its action was bacteriostatic at low concentrations. In general, the *in vitro* activity of the metal complexes are significantly better than those of the free (uncomplexed) ligands, indicating that the metal atom plays a significant role during the interaction of the complex with the microbe.

Recently in our laboratory we have synthesised and structurally characterised copper(II) (McCann *et al.* 1995; Devereux *et al.* 1996; Devereux *et al.* 1998), manganese(II) (Casey *et al.* 1994; McCann *et al.* 1997a,b; Geraghty *et al.* 1998) and molybdenum(II) (Whelan *et al.* 1997) complexes of α,ω-dicarboxylic acids ($HO_2C(CH_2)_nCO_2H$). In this paper we detail the *in vitro* anti-*Candida* and antibacterial (*P. aeruginosa*, *S. aureus* and *E. coli*) activities of copper(II) and selected manganese(II) dicarboxylate complexes.

Methods and materials

$[Cu(O_2C(CH_2)_nCO_2)]{\cdot}xH_2O$ complexes were prepared using a minor modification of the literature method (Asai *et al.* 1959). Details for the synthesis of the butanedioate, butanedioate/1,10-phenanthroline and butanedioate/2,2'-bipyridine complexes (**2**, **10** and **18**, respectively) are outlined below and the other copper(II) complexes in their respective series were prepared in a similar manner. Literature methods were used to synthesise $[Mn(O_2C(CH_2)_3CO_2)]{\cdot}H_2O$ (**25**) (Geraghty *et al.* 1998), $[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_3CO_2)(phen)_2H_2O](O_2C(CH_2)_3\ CO_2){\cdot}12H_2O$ (**28**) (Geraghty *et al.* 1998), $[Mn(O_2C(CH_2)_4CO_2)H_2O]$ (**26**) (McCann *et al.* 1997a), $[Mn(O_2C(CH_2)_4CO_2)(phen)_2H_2O]{\cdot}7H_2O$ (**29**) (McCann *et al.* 1997a), $[Mn(O_2C(CH_2)_5CO_2)]$ (**27**) (McCann *et al.* 1997b), $[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_5CO_2)(phen)_2H_2O](O_2C(CH_2)_5CO_2){\cdot}12.5H_2O$ (**30**) and $[Mn(O_2C(CH_2)_2CO_2)(bipy)\ (H_2\ O)_2]{\cdot}H_2O_n$ (**31**) (McCann *et al.* 1997b). Infrared spectra were recorded as KBr discs in the region 4000–400 cm^{-1} on a Nicolet Impact 400D FT-IR Spectrometer. The spectra of all of the metal complexes contained prominent carboxylate $\nu_{asym}OCO$ and $\nu_{sym}OCO$ stretching bands at *ca.* 1600 cm^{-1} and *ca.* 1400 cm^{-1}, respectively (Nakamoto 1978). Additional characteristic bands were exhibited by the 1,10-phenanthroline (*ca.* 855 cm^{-1} and *ca.* 743 cm^{-1}) and the 2,2'-bipyridine adducts (*ca.* 755 cm^{-1} and *ca.* 620 cm^{-1}) (Nakamoto 1978). Conductivity measurements on the water-soluble complexes were taken at 25 °C using an AGB Scientific Ltd. model 10 conductivity meter. Elemental analysis were carried out by the Microanalytical Laboratory, University College Cork, Ireland.

*$[Cu(O_2C(CH_2)_2CO_2)]$ (**2**)*

To a stirred solution of butanedioic acid (1.77g, 15.0 mmol) in distilled water (*ca.* 150 cm^3) was added

$[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$ (3.0 g, 7.5 mmol). The resulting green-blue suspension was refluxed for 3 h and during this time the condenser was periodically removed to allow some of the liberated acetic acid to escape from the reaction flask (CARE! foaming occurs). The suspension was filtered whilst hot, and the blue product washed with distilled water, ethanol and ether, and then dried *in vacuo*.

*$[Cu(O_2C(CH_2)_2CO_2)(phen)_2]\cdot 2\,H_2O$ (**10**)*
$[Cu(O_2C(CH_2)_2CO_2)]$ (**2**) (1.0 g, 5.6 mmol) and 1,10-phenanthroline hydrate (2.1 g, 10.6 mmol) were refluxed in ethanol (40 cm^3) for 2 h. The resulting green solution was filtered whilst hot and on standing the blue product precipitated. The solid was filtered off, washed with a small volume of ice-cold ethanol and then dried *in vacuo*.

*$[Cu(O_2C(CH_2)_2CO_2)(bipy)_2]\cdot 9\,H_2O$ (**18**)*
$[Cu(O_2C(CH_2)_2CO_2)]$ (1.0 g, 5.6 mmol) (**2**) and 2,2′-bipyridine (1.66 g, 10.6 mmol) were refluxed in an ethanol:water mixture (3:1, 100 cm^3) for 2 h. The resulting blue-green solution was filtered whilst hot and on standing the blue product precipitated. The solid was filtered off, washed with a small volume of ice-cold ethanol and then dried *in vacuo*.

Anti-*Candida susceptibility testing*

C. albicans (three clinical isolates) were obtained from in St. James's Hospital, Dublin, Ireland. One clinical isolate each of the following species *C. krusei, C. tropicalis* and *C. glabrata* were obtained as a gift from Dr. D. O'Sullivan, Dental Hospital, Dublin. The isolates were stored on Sabouraud dextrose agar (SDA) plates at 4 °C.

Solutions of water-soluble test complexes were prepared by dissolving the complex (0.02 g) in distilled water (100 cm^3) to yield a stock solution with a concentration of 200 μg cm^{-3}. The solutions were filter sterilised using a Millipore membrane filter (0.45 μm). Complexes which were insoluble in water and in DMSO were ground to a fine powder (0.02 g) and then suspended in sterile distilled water (100 cm^3). The test complexes for the other *Candida* species (**11–13, 28–30**) were prepared in an identical manner except that the initial stock solution was at a concentration of 1000 μg cm^{-3}.

RPMI-1640 broth medium (Sigma R 7755) was used for the anti-*Candida* susceptibility testing. The medium (1 dm^3) was supplemented with L-glutamine (0.3 g) and morpholinepropanesulfonic acid (MOPS) (34.6 g) and was then adjusted to pH 7.0 using sterile NaOH (0.2 M). The broth macrodilution reference method was used (NCCLS publication M27-P 1979). Prior to testing, yeast cells were grown on Sabouraud dextrose agar (SDA) at 37 °C for 24 h. Cell suspensions were prepared in sterile phosphate buffered saline (5 cm^3) to a density of 0.5 McFarland standard. A 1:100 dilution of these cell suspensions were made in RPMI-1640 medium so that the cell concentration of the final inoculum was 3.5×10^4–5.0×10^5 cells cm^{-3}. The prepared cell suspension (900 μl) was dispensed into sterile test tubes and to this was added the test stock complex solution (100 μl) to yield working solutions of the test complexes of concentration 20 μg cm^{-3} (for *C. albicans*) and 100 μg cm^{-3} (for other *Candida* species). The test tubes were then incubated in a shaking water bath for 24 h at 37 °C with continuous shaking. Each complex was assessed in triplicate and three independent experiments were performed. The resulting nine data points were statistically analysed using ANOVA one-way analysis of variance followed by Tukey's family error rate.

Antibacterial susceptibility testing

The antibacterial agents penicillin, ampicillin, ceftazidime and chloramphenicol were obtained in disc form from Oxoid (in cartridges of 50 discs for use in the Oxoid disc dispenser MK11) and stored at 4 °C. *E. coli* (2 isolates), *P. aeruginosa* (1 isolate) and *S. aureus* (2 isolates), isolated from transplant patients in St. James's Hospital, Dublin, were supplied by Dr. S. McConkey. The isolates were stored on Tryptic soy agar (TSA) plates at 4 °C and were subcultured monthly from the initial stock culture received. Solutions of water-soluble test complexes were prepared by dissolving the complex (0.01 g) in distilled water (100 cm^3) to yield a stock solution with a concentration of 100 μg cm^{-3}. The solutions were filter sterilised using a Millipore membrane filter (0.45 μm). Complexes which were insoluble in water and in DMSO were ground to a fine powder (0.01 g) and suspended in sterile distilled water (100 cm^3).

The Kirby–Bauer method was used for bacterial susceptibility testing (Cooper 1968). Bacteria were grown for 24 h on TSA plates at 37 °C. Each of the five bacterial isolates were touched with a wire loop and transferred to test tubes containing Tryptic Soy Broth (TSB) (5 cm^3). The tubes were incubated for 5 h to produce a suspension which was standardised to

a 1.0 McFarland standard in PBS. Plates were inoculated by dipping a sterile cotton swab into the prepared suspension and removing the surplus by rotation of the swab against the side of the tube. The Mueller Hinton (MH) agar plate (20 cm^3 of MH in a 90 mm diameter petri dish) was inoculated by evenly streaking the swab over the entire surface of the plate. Antibiotic discs containing penicillin (10 μg for *S. aureus*), ampicillin (10 μg for *E. coli*), ceftazidime (30 μg for *P. aeruginosa*) and chloramphenicol (30 μg for *E. coli*) were dispensed onto the agar plates. Blank discs (no antibiotic present) were dispensed onto the remaining agar plates and 100 μl of the stock solutions or suspensions of each test complex was added to each of these discs. The plates were incubated (inverted) for 16–18 h at 37 °C and any visible zone of growth inhibition around the disc was measured using callipers.

Results and discussion

Copper(II) complexes

The dicarboxylate complexes of general formula, $[Cu(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ (n = 1–8; complexes **1**–**8**), are recovered in good yield by reacting $[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$ with the appropriate dicarboxylic acid. These complexes are thought to be essentially isostructural with the structurally characterised polymeric butanedioic acid complex $[Cu_2(O_2C(CH_2)_2CO_2)_2(H_2O)_2]_n$ (O'Connor & Maslen 1966). With the exception of the propanedioate complex, $[Cu(O_2C(CH_2)CO_2]H_2O$ (**1**), the $[Cu(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ complexes were insoluble in water. Furthermore, the low molar conductivity of **1** indicates that the propanedioate ligand remains coordinated to the copper upon dissolution of the complex in water.

The water-soluble 1,10-phenanthroline and 2,2′-bipyridine adducts, $[Cu(O_2C(CH_2)_nCO_2)(phen)_2]\cdot xH_2O$ (complexes **9**-**16**) and $[Cu(O_2C(CH_2)_nCO_2)(bipy)_y]\cdot xH_2O$ (y = 1, 2) (complexes **17**-**24**), respectively, were prepared by treating the parent $[Cu(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ complexes with the appropriate NN-chelating ligand in a *ca.* 1:2 molar ratio. The copper(II) phenanthroline complexes containing heptanedioic acid, $[Cu(O_2C(CH_2)_5CO_2)(phen)_2]\cdot 1\ 1.73H_2O$ (**13**), and octanedioic acid, $[Cu(O_2C(CH_2)_6CO_2)(phen)_2]\cdot 1\ 2H_2O$ (**14**), have previously been characterised by X-ray crystallography (McCann*et al.* 1995). In each case the metal is at the centre of a distorted octahedron comprising four nitrogen atoms from two chelating phenanthroline ligands and two oxygen atoms from a single asymmetric chelating carboxylate function, with the remaining carboxylate group of the diacid uncoordinated. Although none of the present copper(II) 2,2′-bipyridine adducts have yet been structurally characterised the X-ray crystal structure of the polymeric manganese(II) butanedioic acid 2,2′-bipyridine complex $[Mn(O_2C(CH_2)_2CO_2)(bipy)(H_2O)_2]\cdot H_2O_n$ (**31**) has been reported (see below).

With the exception of the propanedioate complexes, **9** and **17**, the high molar conductivity values recorded for aqueous solutions of the phenanthroline and bipyridine adducts indicates that the diacid ligand dissociates from the metal upon dissolution of these complexes in water, and that formation of the dications $[Cu(phen)_2(H_2O)_n]^{2+}$ and $[Cu(bipy)_2(H_2O)_n]^{2+}$ occurs (the mono-bipyridine complex **19** presumably dissociates to form $[Cu(bipy)(H_2O)_n]^{2+}$). For complexes **9** and **17** dissociation of the propanedioate ligand from the metal is less extensive, indicating that this short chain diacid ligand is probably forming a relatively stable six-membered chelate ring system by coordinating a carboxylate oxygen atom from opposite ends of the diacid chain to the metal.

Unlike their copper(II) analogues the manganese(II) complexes of formula $[Mn(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ (n = 3–5) (complexes **25**–**27**) were water-soluble and were extensively dissociated into $[Mn(H_2O)_6]^{2+}$ and $(O_2C(CH_2)_nCO_2)^{2-}$ ions in aqueous solution. The water-soluble phenanthroline and bipyridine adducts (complexes **28**–**30** and **31**, respectively) were prepared in a similar fashion to that described above for the copper(II) complexes and all four have previously been structurally characterised. In contrast to the mononuclear copper phenanthroline complexes, $[Cu(O_2C(CH_2)_3CO_2)(phen)_2]\cdot 6\ H_2O$ (**11**) and $[Cu(O_2C(CH_2)_5CO_2)(phen)_2]\cdot 1\ 1.73H_2O$ (**13**) (McCann *et al.* 1995), the respective manganese complexes $[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_3CO_2)(phen)_2H_2O](O_2C(CH_2)_3CO_2)\cdot 12H_2O$ (**28**) (Geraghty *et al.* 1998) and $[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_5CO_2)(phen)_2H_2O](O_2C(CH_2)_5CO_2)\cdot 12.5H_2O$ (**30**) (McCann *et al.* 1997a) each comprise distinct $[Mn(phen)_2(H_2O)_2]^{2+}$ and $(O_2C(CH_2)_nCO_2)^{2-}$ ions together with the neutral moiety $[Mn(O_2C(CH_2)_nCO_2)(phen)_2H_2O]$ in the solid state. In complex **28** the dication $[Mn(phen)_2(H_2O)_2]^{2+}$ is coordinated to four nitrogen atoms from two chelating phenan-

throline ligands and two oxygen atoms from two *cisoid* water molecules. In the neutral fragment $[Mn(O_2C(CH_2)_3CO_2)(phen)_2(H_2O)]$ the metal atom is coordinated to four nitrogen atoms from two chelating phenanthroline ligands, one oxygen atom from a unidentate $(O_2C(CH_2)_3CO_2)^{2-}$ ligand and one oxygen atom from a water molecule which is *cis* with respect to the coordinated carboxylate oxygen of the pentanedioate ligand. Complex **30** is isostructural with **28** (McCann *et al.* 1997a). When dissolved in water it is likely that the neutral moiety $[Mn(O_2C(CH_2)_nCO_2)(phen)_2(H_2O)]$ dissociates to form the stable dication $[Mn(phen)_2(H_2O)_2]^{2+}$ and the dianion $(O_2C(CH_2)_nCO_2)^{2-}$.

The bipyridine complex $[Mn(O_2C(CH_2)_2CO_2)(bipy)(H_2O)_2]\cdot H_2O_n$ (**31**) consists of infinite chains in which pairs of symmetry related metal atoms are bridged by butanedioic dianionic ligands, the carboxylate functions of which coordinate to the metals in a unidentate fashion (McCann *et al.* 1997b). Each manganese atom has a distorted octahedral coordination geometry and is ligated by a chelating bipyridyl ligand, two *cisoid* water molecules and two *cisoid* carboxylate oxygen atoms from separate bridging butanedioic ligands. The complex behaves as a strong electrolyte in water with formation of $[Mn(bipy)(H_2O)_4]^{2+}$ and $(O_2C(CH_2)_2CO_2)^{2-}$.

Anti-Candida activity

The activity of the uncoordinated ligands, some simple copper(II) and manganese(II) salts and the metal complexes on *C. albicans* were each assessed at a concentration of 20 μg cm^{-3} (Table 1). The free dicarboxylic acids had no significant activity on the growth of the isolates with the exception of propanedioic acid (isolate 1 and isolate 3, 50% and 58% growth, respectively).

Whereas the simple salts $[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$, $Mn(O_2CCH_3)_2\cdot 4H_2O$ and $MnCl_2\cdot 4H_2O$ had no effect on the growth of the isolates $CuSO_4\cdot 5H_2O$ and $CuCl_2\cdot 2H_2O$ exhibited moderate activity against isolate 3. The insoluble complexes $[Cu(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ (n = 1–8; complexes **1**–**8**) were essentially ineffective at preventing the growth of isolate 1. However, complexes **3**–**7** had reasonable activity against isolate 2 and, in addition, complexes **6** and **7** also showed moderate activity against isolate 3. The water-soluble manganese complexes $[Mn(O_2C(CH_2)_nCO_2)]\cdot xH_2O$ (n = 3–5; complexes **25**-**27**) were inactive.

Metal-free 1,10-phenanthroline and all of the copper(II) and manganese(II) phenanthroline complexes (complexes **9**–**16** and **28**–**30**, respectively) were potent growth inhibitors of all three isolates of *C. albicans*. In general, the activity of the complexes was independent of the chain length of the dicarboxylate ligand indicating that the active drug species is the $[M(phen)_2(H_2O)_n]^{2+}$ dication (M = Cu, Mn). Similar results have previously been found for the manganese(II) and copper(II) dicarboxylate/1,10-phenanthroline complexes $[Mn(bdoa)(phen)_2]\cdot H_2O$, $[Cu_2(bdoa)(phen)_4]bdoa\cdot 13H_2O$ ($bdoaH_2$ = benzene-1,2-dioxyacetic acid) (Geraghty *et al.* 1999).

In contrast to the remarkable activity of 1,10-phenanthroline and its metal complexes metal-free 2,2′-bipyridine and the majority of its metal complexes had no inhibitory effect on the growth of the three isolates of *C. albicans*. $[Cu(O_2C(CH_2)CO_2)(bipy)_2]\cdot 2H_2O$ (**17**) was the only 2,2′-bipyridine complex which showed a pronounced enhancement of activity when compared to both uncomplexed 2,2′-bipyridine and its carboxylate precursor complex $[Cu(O_2C(CH_2)CO_2)]$ (**1**). Given the structural similarities of the solution dications, $[Cu(phen)_2(H_2O)_n]^{2+}$ and $[Cu(bipy)_2(H_2O)_n]^{2+}$, and also the fact that the stability constants of $[Cu(phen)_2]^{2+}$ and $[Cu(bipy)_2]^{2+}$ are almost the same ($\log\beta$ = 21 and 17, respectively) (Albert 1973), it might have been anticipated that their anti-*Candida* activities would have been quite similar. Indeed, the dissimilarity in the performance of the two dications prompts the question as to the mode of action of $[Cu(phen)_2(H_2O)_n]^{2+}$. If the potency of $[Cu(phen)_2(H_2O)_n]^{2+}$ was merely as a direct consequence of adherence of the dication to the exterior of the cell wall (through chemical interaction of the metal with nucleophilic functional groups on the wall surface) then the activities of $[Cu(phen)_2(H_2O)_n]^{2+}$ and $[Cu(bipy)_2(H_2O)_n]^{2+}$ would have been expected to have been quite similar. The same arguments can be applied to $[Mn(phen)_2(H_2O)_2]^{2+}$ and $[Mn(bipy)(H_2O)_4]^{2+}$. It is possible that the differences in the activities of the $[M(phen)_2(H_2O)_n]^{2+}$ and $[M(bipy)_2(H_2O)_n]^{2+}$ cations against *C. albicans* is greatly influenced by differences in the abilities of these two dications to traverse the cell wall. It is expected that the more extensive aromatic ring system of the 1,10-phenanthroline ligand, as compared to 2,2′-bipyridine, would confer greater lipophilicity on $[M(phen)_2(H_2O)_n]^{2+}$ and enable it to pene-

Table 1. Anti-*Candida albicans* activity.

Test complex	Yeast isolate		
	C. albicans 1	*C. albicans* 2	*C. albicans* 3
Control	100	100	100
$HO_2C(CH_2)CO_2H$	50 ± 6	95 ± 5	58 ± 2
$HO_2C(CH_2)_2CO_2H$	91 ± 7	93 ± 5	100 ± 5
$HO_2C(CH_2)_3CO_2H$	87 ± 6	94 ± 3	107 ± 6
$HO_2C(CH_2)_4CO_2H$	100 ± 10	106 ± 4	92 ± 9
$HO_2C(CH_2)_5CO_2H$	95 ± 5	97 ± 4	93 ± 5
$HO_2C(CH_2)_6CO_2H$	91 ± 9	101 ± 4	96 ± 8
$HO_2C(CH_2)_7CO_2H$	93 ± 8	103 ± 5	94 ± 7
$HO_2C(CH_2)_8CO_2H$	73 ± 6	75 ± 7	98 ± 11
$[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$	108 ± 9	89 ± 5	92 ± 7
$CuSO_4{\cdot}5H_2O$	84 ± 11	63 ± 1	47 ± 4
$CuCl_2{\cdot}2H_2O$	81 ± 12	84 ± 1	49 ± 5
$[Cu(O_2C(CH_2)CO_2]{\cdot}H_2O$ (**1**)	68 ± 9	64 ± 6	85 ± 7
$[Cu(O_2C(CH_2)_2CO_2]$ (**2**)	86 ± 6	63 ± 8	61 ± 7
$[Cu(O_2C(CH_2)_3CO_2]$ (**3**)	88 ± 5	54 ± 7	77 ± 5
$[Cu(O_2C(CH_2)_4CO_2]$ (**4**)	96 ± 3	53 ± 1	82 ± 4
$[Cu(O_2C(CH_2)_5CO_2]$ (**5**)	91 ± 3	47 ± 3	70 ± 7
$[Cu(O_2C(CH_2)_6CO_2]$ (**6**)	81 ± 4	34 ± 1	54 ± 11
$[Cu(O_2C(CH_2)_7CO_2]$ (**7**)	87 ± 2	45 ± 9	51 ± 8
$[Cu(O_2C(CH_2)_8CO_2]$ (**8**)	78 ± 6	73 ± 8	84 ± 8
$[Cu(O_2C(CH_2)CO_2(phen)_2]{\cdot}H_2O$ (**9**)	18 ± 4	22 ± 9	17 ± 5
$[Cu(O_2C(CH_2)_2CO_2(phen)_2]{\cdot}2H_2O$ (**10**)	11 ± 6	10 ± 3	15 ± 1
$[Cu(O_2C(CH_2)_3CO_2(phen)_2]{\cdot}6H_2O$ (**11**)	5 ± 0.36	10 ± 0.3	5 ± 0.3
$[Cu(O_2C(CH_2)_4CO_2(phen)_2]{\cdot}6H_2O$ (**12**)	15 ± 3	16 ± 3	12 ± 2
$[Cu(O_2C(CH_2)_5CO_2(phen)_2]{\cdot}11.73H_2O$ (**13**)	6 ± 2	10 ± 3	5 ± 0.2
$[Cu(O_2C(CH_2)_6CO_2(phen)_2]{\cdot}12H_2O$ (**14**)	9 ± 2	12 ± 3	6 ± 2
$[Cu(O_2C(CH_2)_7CO_2(phen)_2]{\cdot}6H_2O$ (**15**)	4 ± 1	5 ± 1	4 ± 0.5
$[Cu(O_2C(CH_2)_8CO_2(phen)_2]{\cdot}8H_2O$ (**16**)	4 ± 1	1 ± 0.3	3 ± 1
$[Cu(O_2C(CH_2)CO_2(bipy)_2]{\cdot}2H_2O$(**17**)	31 ± 6	18 ± 5	33 ± 2
$[Cu(O_2C(CH_2)_2CO_2(bipy)_2]{\cdot}9H_2O$ (**18**)	84 ± 10	64 ± 6	70 ± 11
$[Cu(O_2C(CH_2)_3CO_2(bipy)]{\cdot}3H_2O$(**19**)	78 ± 7	81 ± 11	95 ± 6
$[Cu(O_2C(CH_2)_4CO_2(bipy)_2]{\cdot}H_2O$ (**20**)	74 ± 6	104 ± 9	104 ± 7
$[Cu(O_2C(CH_2)_5CO_2(bipy)_2]{\cdot}6H_2O$ (**21**)	87 ± 4	77 ± 5	45 ± 16
$[Cu(O_2C(CH_2)_6CO_2(bipy)_2]{\cdot}6H_2O$ (**22**)	72 ± 8	80 ± 5	84 ± 7
$[Cu(O_2C(CH_2)_7CO_2(bipy)_2]{\cdot}4H_2O$ (**23**)	73 ± 9	92 ± 11	92 ± 10
$[Cu(O_2C(CH_2)_8CO_2(bipy)_2]{\cdot}5H_2O$ (**24**)	91 ± 10	87 ± 8	91 ± 7
1,10-phenanthroline	10 ± 2	4 ± 1	11 ± 1
2,2′-bipyridine	75 ± 6	80 ± 4	104 ± 1
$[Mn(O_2C(CH_2)_3CO_2)]{\cdot}H_2O$ (**25**)	98 ± 10	88 ± 12	93 ± 11
$[Mn(O_2C(CH_2)_4CO_2)]{\cdot}H_2O$ (**26**)	95 ± 7	90 ± 7	87 ± 3
$[Mn(O_2C(CH_2)_5CO_2)]$ (**27**)	81 ± 9	83 ± 6	100 ± 5
$MnCl_2.4H_2O$	92 ± 5	94 ± 5	106 ± 5
$Mn(O_2CCH_3)_2.4H_2O$	85 ± 8	94 ± 5	102 ± 8
$[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_3CO_2)(phen)_2H_2O](O_2C(CH_2)_3CO_2){\cdot}12H_2O$ (**28**)	4 ± 1	3 ± 0.5	6 ± 2
$[Mn(O_2C(CH_2)_4CO_2)(phen)_2(H_2O)]{\cdot}7H_2O$ (**29**)	15 ± 4	14 ± 4	12 ± 3
$Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_5CO_2)(phen)_2(H_2O)]O_2C(CH_2)_5CO_2{\cdot}12.5H_2O$ (**30**)	3 ± 1	5 ± 1	1 ± 0.3
$[Mn(O_2C(CH_2)_2CO_2)(bipy)(H_2O)_2]{\cdot}H_2O_n$ (**31**)	61 ± 19	100 ± 9	100 ± 7

Compounds were tested at a concentration of 20 μg cm^{-3} of aqueous RPMI medium. Complexes **2** - **8** 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 added compound).

Table 2. Effect of selected complexes on the growth of *C. tropicalis, C. kreusi, C. glabrata*

Test complex	Yeast isolate		
	C. tropicalis	*C. kreusi*	*C. glabrata*
$[Cu(O_2C(CH_2)_3CO_2(phen)_2]\cdot 6H_2O$ (**11**)	5 ± 2	15 ± 5	4 ± 1
$[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_3CO_2)(phen)_2H_2O](O_2C(CH_2)_3CO_2)\cdot 12H_2O$ (**28**)	0	0	0
$[Cu(O_2C(CH_2)_4CO_2(phen)_2]\cdot 6H_2O$ (**12**)	3 ± 0.1	12 ± 2	3 ± 0.3
$[Mn(O_2C(CH_2)_4CO_2)(phen)_2(H_2O)]\cdot 7H_2O$ (**29**)	$3 \pm$	12 ± 1	4 ± 0.3
$[Cu(O_2C(CH_2)_5CO_2(phen)_2]\cdot 11.73H_2O$ (**13**)	4 ± 0.2	21 ± 4	2 ± 0.2
$[Mn(phen)_2(H_2O)_2][Mn(O_2C(CH_2)_5CO_2)(phen)_2(H_2O)]O_2C(CH_2)_5CO_2\cdot 12.5H_2O$ (**30**)	0	0	0
1,10-phenanthroline	5 ± 1	8 ± 2	12 ± 2

Compounds were tested at a concentration of 100 μg cm^{-3} of aqueous RPMI medium. Yeast cells of the three species 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 added compound).

trate the cell wall and promote adverse intracellular interactions.

When assessing the performance of 1,10-phenanthroline and its metal complexes against *C. albicans* it is worthwhile considering the ratio of drug molecule: yeast cell at the start of the 24 h incubation period. For metal-free 1,10-phenanthroline the ratio is *ca.* 1×10^{12} molecules for each *Candida* cell whilst, for example, for $[Cu(O_2C(CH_2)_8CO_2(phen)_2]\cdot 8H_2O$ (**16**) the ratio is *ca.* 3 x 10^{11} complex molecules for each yeast cell. Furthermore, given that the total amount of copper present naturally in a particular isolate of *C. albicans* has been reported to be 7.3 ppm Pijck (1969) it is reasonable to assume that some of this copper (and indeed other loosely bound transition metal ions present in the yeast cell) is sequestered by the administered metal-free 1,10-phenanthroline, and that the resultant metal-phenanthroline complex is the effective drug.

A selection of complexes (**11**–**13**, **28**–**30**) which were most active against *C. albicans* were tested against *C. glabrata, C. tropicalis* and *C. kreusi* (Table 2). The manganese(II) complexes (**28**–**30**) retained their superior activity when compared to the copper(II) complexes (**11**–**13**). The most noticeable difference in activity between the two families is the increased activity of the manganese(II) complexes against *C. kreusi* compared to the copper(II) complexes. Metal-free 1,10-phenanthroline is also highly active against these three *Candida* species.

Antibacterial activity

Antibacterial studies were conducted using selected metal complexes (**11**–**14**, **28**–**30**) and also metal-free 1,10-phenanthroline. Clinical isolates of the gram-negative bacterium *E. coli* and the gram-positive bacterium *S. aureus* were resistant to all of the metal complexes and also to metal-free 1,10-phenanthroline. Interestingly, only the copper phenanthroline complexes (**11**–**14**) showed intermediate activity (in comparison to ceftazidime) against gram-negative *P. aeruginosa*, with 1,10-phenanthroline and the remainder of the copper and manganese complexes being inactive.

Conclusions

The metal-phenanthroline complexes and metal-free 1,10-phenanthroline exhibit broad-spectrum anti-*Candida* activity. In contrast, anti-bacterial activity was confined to the copper phenanthroline complexes against *P. aeruginosa*. The observed variation in the activity of the metal-phenanthroline complexes and metal-free 1,10-phenanthroline across the various classes of organisms studied may be attributable to differences in cell wall and/or membrane construction (gram-positive bacteria; peptidoglycan and teichoic acid; gram-negative bacteria; peptidoglycan and liposaccharide; *Candida* species, chitin, mannoproteins and other polysaccharides) (Brock & Madigan 1991).

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