Supplementary Material (ESI) for Dalton Transactions



# Supporting Information

Accompanying the manuscript

# Bis-Phenanthroline Copper(II) Phthalate Complexes are Potent *in vitro* Antitumour Agents with 'Self-Activating' Metallo-Nuclease and DNA Binding Properties

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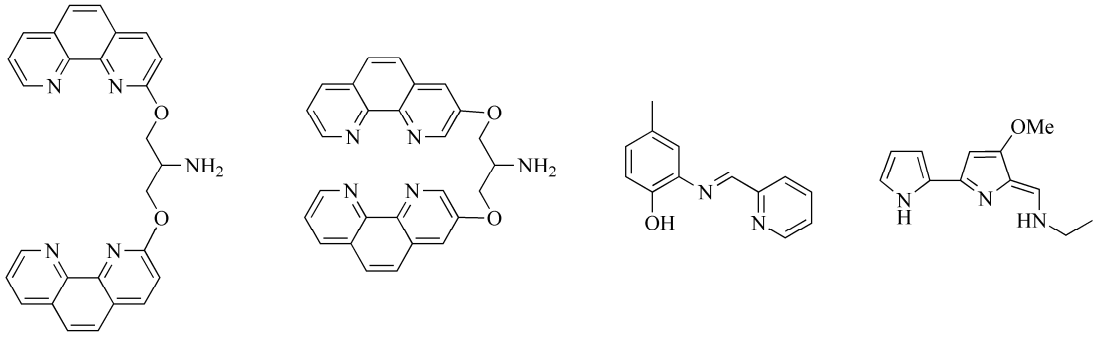


2-Clip-Phen 3-Clip-Phen HPyrimol tambjamine E

***Figure 1:*** Ligand structures of 2-clip-phen, 3-clip-phen, HPyarmol and tambjamine E

**S-1. Synthesis of the Cu(II) complexes 1-6.**

**Materials.** Chemicals, CT-DNA and duplex polymers were purchased from Sigma Aldrich (Ireland) and used without further purification. Supercoiled plasmid DNA (pUC18) was supplied by Roche Diagnostics Ltd (UK).

**Infrared Spectroscopy.** Solid-state IR were recorded in the region 4000 - 400 $cm^{-1}$ on a Nicolet FT-IR 5DXB infrared spectrometer with solid samples firstly being finely ground then mixed with KBr. Pellets consisted of 5 mg of analyte per 195 mg of KBr.

**Raman Spectroscopy.** Raman spectra were recorded from saturated potassium bromide pellets on an Instruments S.A. Labram 1B spectrometer with a confocal Raman imaging microscope system equipped with an Argon ion (514.5 nm, 50 *m*W) laser source. The light was imaged to a diffraction limited spot via the objective of an Olympus BX40 microscope and spectra were recorded over the range of 4000-150 $cm^{-1}$. The accumulation time was optimized for intensity; typically ten accumulations were recorded for each spectral window.

**Magnetic Susceptibility.** Measurements were made using a Johnson Matthey Magnetic Susceptibility balance. $[HgCo(SCN)_4]$ was used as a reference.

**UV-vis Spectroscopy**. Samples were recorded in both solution and solid-state spectra using a Varian Cary 50 Scan single beam spectrophotometer which covered the range 800-190 nm using a Xenon lamp as light source. Solid-state samples were generated by finely grinding samples of each complex then drop-casting from a chloroform suspension onto a glass substrate. Solution-state spectra were recorded at 4.0 m*M* in DMSO.

**Conductivity**. Molar conductivity measurements were made at 25$^{o}$C using an Orion 150 Aplus conductivity meter at 4.0 m*M* in DMSO.

**Elemental Analysis.** Microanalytical data for the complexes were reported by the Microanalytical Laboratory, University College Dublin, Ireland. Samples **1-6** were difficult to balance in terms of % hydrogen. The complexes all lost weight due to lattice molecules of water and ethanol being evaporated. We can account for water and ethanol molecules in the lattice from the IR and raman spectra of **1-6,** but some disparity does exists, in terms of molecules of hydration, between the X-ray and microanalytical results.

**Synthesis.** Complexes $\{[Cu(ph)(H_2O)]\}_n$ (**1**), $\{[Cu(isoph)(H_2O)_3]2H_2O\}_n$ (**2**) and $\{[Cu(tereph)(H_2O)_3]2H_2O\}_n$ (**3**) were synthesised according to the following general procedure; to a hot solution of the relevant phthalic acid (7.5 g, 45 mmol) and sodium hydroxide (3.16 g, 80 mmol) in water (150 $cm^3$) was added copper(II) chloride dihydrate (3.0 g, 22.31 mmol) with resultant blue solution being refluxed for 3 hr. The blue powder which deposited was filtered, washed with a small volume of ethanol and air-dried. $\{[Cu(ph)(H_2O)]\}_n$ (**1**); Yield: 4.62 g (81.33 %). % Calc: C, 40.60; H, 2.13 % Found: C, 40.49; H 2.43. IR (KBr): 456, 502, 574, 600, 658, 704, 717, 769, 798, 823, 834, 863, 887, 970, 999, 1034, 1086, 1144, 1160, 1262, 1298, 1375, 1444, 1486, 1571, 1592, 1613, 1638, 1855, 1889, 1933, 1964, 1995, 2134,

2235, 2531, 2741, 3064, 3461, 3512.95 $cm^{-1}$. Raman (KBr): 181, 235, 278, 317, 397, 463, 562, 598, 657, 721, 765, 798, 835, 1037, 1084, 1160, 1298, 1298, 1367, 1408, 1445, 1491, 1588, 1611, 3067, 3462 $cm^{-1}$. Solubility: DMSO/DMF. $\mu_{eff}$ = 1.47 B.M. UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 608 nm, UV-Vis (DMSO): $\lambda_{d\text{-}d}$ = 608 nm, ε = 27 $dm^3$ $mol^{-1}$ $cm^{-1}$, Λ*M* (DMSO): 27.30 S $cm^2$ $mol^{-1}$, $\{[Cu(isoph)(H_2O)_3]2H_2O\}_n$ (**2**); Yield: 13.3 g (93.86 %). % Calc: C, 30.24; H, 4.44 % Found: C, 30.56; H 2.18. IR (KBr): 449, 477, 554, 660, 735, 800, 821, 928, 1002, 1077, 1102, 1162, 1177, 1273, 1313, 1327, 1385, 1444, 1480, 1565, 1612, 1685, 2562, 2674, 3396, 3591 $cm^{-1}$. Raman (KBr): 200, 463, 578, 659, 697, 726, 801, 825, 1004, 1181, 1293, 1409, 1457, 1573, 1611, 3075 $cm^{-1}$. Solubility: DMSO/DMF. $\mu_{eff}$ = 1.87 B.M. UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 671 nm, UV-Vis (DMSO): $\lambda_{d\text{-}d}$ = 725 nm, ε = 145 $dm^3$ $mol^{-1}$ $cm^{-1}$, Λ*M* (DMSO): 16.82 S $cm^2$ $mol^{-1}$, $\{[Cu(tereph)(H_2O)_5]\}_n$ (**3**); Yield: 9.76 g (93.86 %). % Calc: C, 30.24; H, 4.44 % Found: C, 30.56; H 2.18. IR (KBr): 449, 477, 554, 660, 735, 800, 821, 928, 1002, 1077, 1102, 1162, 1177, 1273, 1313, 1327, 1385, 1444, 1480, 1565, 1612, 1685, 2562, 2674, 3396, 3591 $cm^{-1}$. Raman (KBr): 200, 463, 578, 659, 697, 726, 801, 825, 1004, 1181, 1293, 1409, 1457, 1573, 1611, 3075 $cm^{-1}$. Solubility: forms only suspensions in DMSO/DMF. $\mu_{eff}$ = 1.80 B.M. UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 636 nm.

Complexes $[Cu(ph)(phen)_2]3H_2O.2EtOH$ (**4**), $[Cu(isoph)(phen)_2].5H_2O.EtOH.0.5MeOH$ (**5**) and $[\{Cu\{(phen)_2\}_2(terph)](terph)13.5H_2O.2EtOH$ (**6**) were synthesised according to the general procedure; solutions of **1**, **2** or **3** (2.0 mmol) in ethanol (50 $cm^3$) were treated with 1,10-phenanthroline (0.73 g, 4.0 mmol) with resultant solutions being refluxed for 3 hr. In the synthesis of **4** and **6** blue powders deposited which were filtered and the resulting filtrates were allowed to stand for 1 week and 1 month, respectively, resulting in the formation of the corresponding crystals suitable for X-ray crystallography. In the synthesis of **5**, a clear blue solution resulted, allowed to stand for 1 week resulting in the formation of crystals suitable for X-ray crystallography. $[Cu(ph)(phen)_2]3H_2O.2EtOH$ (**4**); Yield: 0.40 g (27.99 %), % Calc: C, 58.89; H, 5.22; N, 7.63, % Found: C, 52.45; H, 2.87; N, 7.50 IR (KBr): 410, 622, 694, 738, 778, 853, 987, 1090, 1137, 1216, 1345, 1421, 1504, 1504, 1561, 1586, 1615, 3058, 3765 $cm^{-1}$. Raman (KBr): 246, 410, 551, 709, 1053, 1097, 1276, 1295, 1405, 1447, 1503, 1563, 1588, 3065 $cm^{-1}$. Solubility: forms only suspensions in DMSO/DMF. $\mu_{eff}$ = 1.80 B.M. UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 682 nm, $[Cu(isoph)(phen)_2].5H_2O.EtOH.0.5MeOH$ (**5**); Yield: 0.07 g (5.47 %), % Calc: C, 55.98; H, 5.17; N, 7.57, % Found: C, 56.80; H, 3.33; N, 8.17 IR (KBr): 431, 647, 723, 748, 816, 853, 902, 942, 996, 1067, 1103, 1143, 1223, 1263, 1308, 1314, 1356, 1379, 1426, 1450, 1472, 1493, 1518, 1577, 1604, 3060, 3405 $cm^{-1}$. Raman (KBr): 190, 309, 429, 560, 739, 817, 1002, 1054, 1255, 1305, 1343, 1370, 1430, 1455, 1518, 1585, 1606, 1626, 3078 $cm^{-1}$. Solubility: DMSO/DMF. $\mu_{eff}$ = 2.02 B.M., UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 638 nm, UV-Vis (DMSO): $\lambda_{d\text{-}d}$ = 675 nm, ε = 35 $dm^3$ $mol^{-1}$ $cm^{-1}$, Λ*M* (DMSO): 2.25 S $cm^2$ $mol^{-1}$, $[\{Cu\{(phen)_2\}_2(terph)](terph)13.5H_2O.2EtOH$ (**6**); Yield: 0.78 g (52.19 %). % Calc: C, 54.03; H, 5.27; N, 7.41, % Found: C, 56.11; H, 3.19; N, 7.30. IR (KBr): 429, 452, 507, 546, 593, 647, 722, 743, 783, 825, 850, 868, 887, 1013, 1031, 1050, 1089, 1105, 1144, 1197, 1224, 1309, 1344, 1374, 1385, 1427, 1498, 1517, 1569, 1602, 1625, 1693, 3057, 3388$cm^{-1}$. Raman (KBr): 274, 312, 436, 567, 611, 744, 865, 1062, 1137, 1256, 1319, 1436, 1523, 1614, 3082 $cm^{-1}$. Solubility: forms only suspensions in DMSO/DMF. $\mu_{eff}$ = 1.97 B.M. UV-Vis (Solid-State): $\lambda_{d\text{-}d}$ = 575 nm, 700 nm (sh).

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**S-2. X-ray crystallographic data for 4-6.**

Complexes **4-6** were characterised by single X-ray diffraction as $[Cu(ph)(phen)_2]3H_2O.2EtOH$, $[Cu(isoph)(phen)_2].5H_2O.EtOH.0.5MeOH$ and $[\{Cu\{(phen)_2\}_2(terph)](terph)13.5H_2O.2EtOH$ respectively. For complexes **4** and **5** single crystal X-ray diffraction data were collected on a Bruker X8 APEX2 CCD diffractometer (Bruker 2004, APEX2 Version 2.1 Bruker AXS Inc., Madison, Wisconsin, USA) using the APEX2 suite of programs and further analysed using PLATON.[1] Water molecule hydrogen atoms were located via the program CALC-OH.[2] Data for complex **6** were collected at 153(2) K using a Siemens P4 diffractometer and corrected for Lorentz, polarisation and absorption effects. The structure was solved by direct methods and refined by full matrix least-squares on $F^2$. The asymmetric unit contains half a cation, half a terephthalate dianion, six well-ordered water molecules and a disordered solvate region. The disorder was modelled as containing an ethanol molecule distributed over three sites (50:25:25) and a further 75% occupancy water molecule, also disordered over two sites (50:25). All the non-hydrogen atoms were refined with anisotropic atomic displacement parameters, except for the disordered solvate at 25% occupancy. Hydrogen atoms bonded to full-occupancy carbon atoms were inserted at calculated positions, those bonded to oxygen atoms were located from difference Fourier maps and refined under restraints, H atoms bonded to 25% occupancy oxygen atoms were not included. All programs used in the structure solutions and refinements are contained in the SHELXTL package.[3,4]

***Table 1:*** Crystal data and refinement for compounds **4** - **6**.

| Compound | **4** | **5** | **6** |
|---|---|---|---|
| Empirical formula | $C_{36}H_{38}N_4O_9Cu$ | $C_{34.5}H_{38}N_4O_{10.5}Cu$ | $C_{68}H_{79}Cu_2N_8O_{23.50}$ |
| Formula weight | 734.24 | 740.23 | 1511.47 |
| Temperature (K) | 100(2) | 100(2) | 153(2) |
| Wavelength(Å) | 0.71073 | 0.71073 | 0.71073 |
| Crystal system | Triclinic | Triclinic | Triclinic |
| Space group | P-1 | P-1 | P-1 |
| a (Å) | 11.335(6) | 10.9068(7) | 12.598(2) |
| b (Å) | 12.183(6) | 11.8165(8) | 12.866(2) |
| c (Å) | 14.539(7) | 14.7457(10) | 13.330(3) |
| α ($^o$) | 113.97(2) | 74.650(4) | 100.665(14) |
| β ($^o$) | 92.95(3) | 79.904(3) | 108.868(15) |
| γ ($^o$) | 108.81(3) | 68.909(2) | 107.973(12) |
| Volume (Å$^3$) | 1697.5(14) | 1703.1(2) | 1844.7(6) |
| Z | 2 | 2 | 1 |
| Density (calc) (Mg/m$^3$) | 1.436 | 1.443 | 1.361 |
| Absorption coeff (mm$^{-1}$) | 0.706 | 0.707 | 0.657 |
| F(000) | 766 | 772 | 789 |
| Crystal size (mm$^3$) | 0.52 x 0.40 x 0.38 | 0.26 x 0.18 x 0.15 | 0.75 x 0.40 x 0.18 |
| q range ($^o$) | 1.97 - 32.44 | 2.26 - 28.39 | 2.48 - 25.00 |
| Index ranges | -16<=h<=15<br>-18<=k<=17<br>-21<=l<=17 | -14<=h<=14<br>-15<=k<=15<br>-19<=l<=19 | 0<=h<=14<br>-14<=k<=13<br>-14<=l<=14 |
| Reflections collected | 33406 | 53727 | 6627 |
| Independent refl [$R_{int}$ ] | 10772 [0.0363] | 8301[0.0283] | 6314 [ 0.0168] |
| Completeness to $\theta$ (=25.00$^o$) | 98.8% | 98.4 % | 97.4% |
| Absorption correction | Semi-empirical | Semi-empirical | Semi-empirical from equivalents |
| Refinement method | Full-matrix | Full-matrix | Full-matrix |
| Data/restraints /parameter | 10772 / 9 / 473 | 8301 / 25 / 499 | 6314 / 49 / 505 |
| Goodness-of-fit on F2 | 1.032 | 0.964 | 1.081 |
| Final $R$ indices [$I>2\sigma(I)$] | $R_1$ = 0.0381<br>$wR_2$ = 0.0913 | $R_1$ = 0.0420<br>$wR_2$ = 0.1185 | $R_1$ = 0.0592<br>$wR_2$ = 0.1583 |
| $R$ indices (all data) | $R_1$ = 0.0543<br>$wR_2$ = 0.0980 | $R_1$ = 0.0477<br>$wR_2$ = 0.1231 | $R_1$ = 0.0814<br>$wR_2$ = 0.1713 |
| Largest diff. peak and hole (e.Å$^{-3}$) | 0.595 and -0.509 | 1.028 and -0.967 | 1.204 and -0.394 |

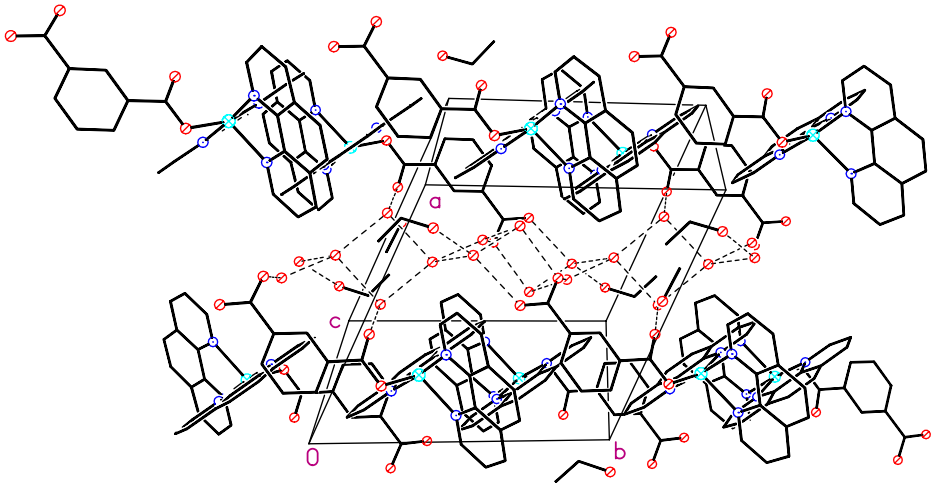


***Figure 2:*** Packing diagram for [Cu(ph)(phen)$_2$]3H$_2$O.2EtOH (**4**)

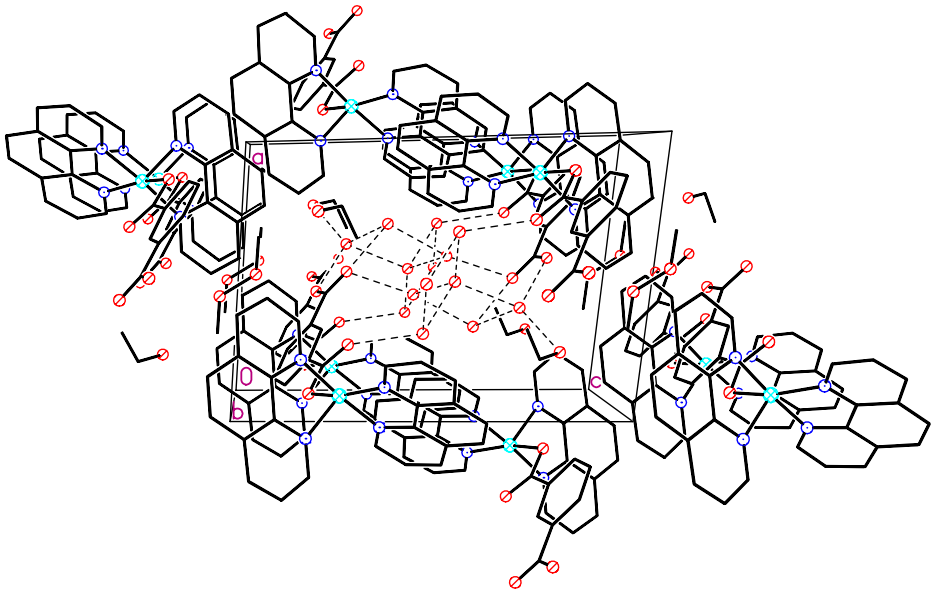


***Figure 3:*** Packing diagram for [Cu(isoph)(phen)$_2$].5H$_2$O.EtOH.0.5MeOH (5)

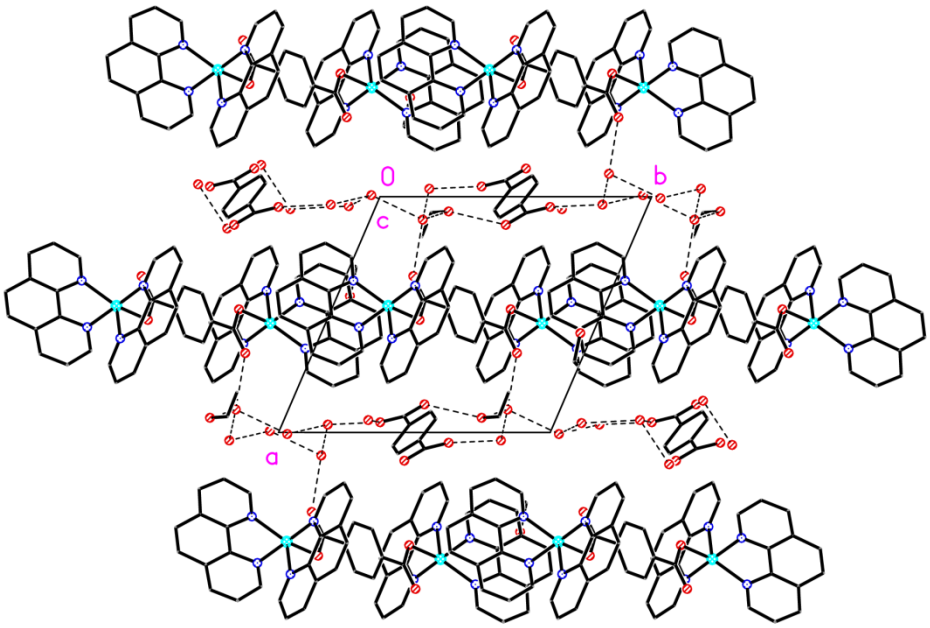


***Figure 4:*** Packing diagram for [{Cu(phen)$_2$}$_2$(terph)](terph)13.5H$_2$O.2EtOH (**6**) Showing the π–π stacking of the phenanthroline groups and the H-bonded network involving the solvate molecules and uncoordinated terph.

***Table 2:*** Selected bond lengths [Å] and angles [°] for $[Cu(ph)(phen)_2]3H_2O.2EtOH$ (**4**)

| | | | |
|---|---|---|---|
| Cu(1)-O(1) | 1.9961(12) | Cu(1)-N(22) | 2.0004(15) |
| Cu(1)-N(11) | 2.0069(15) | Cu(1)-N(21) | 2.0824(16) |
| Cu(1)-N(12) | 2.2060(17) | Cu(1)-O(2) | 2.4930(18) |
| | | | |
| O(1)-Cu(1)-N(11) | 94.65(6) | N(22)-Cu(1)-N(11) | 172.44(5) |
| O(1)-Cu(1)-N(21) | 152.58(5) | N(22)-Cu(1)-N(21) | 81.21(6) |
| N(11)-Cu(1)-N(21) | 95.26(6) | O(1)-Cu(1)-N(12) | 119.26(5) |
| N(22)-Cu(1)-N(12) | 93.21(6) | N(11)-Cu(1)-N(12) | 79.91(6) |
| N(21)-Cu(1)-N(12) | 87.67(6) | | |

***Table 3:*** Selected bond lengths [Å] and angles [°] for $[Cu(isoph)(phen)_2].5H_2O.EtOH.0.5MeOH$ (**5**)

| | | | |
|---|---|---|---|
| Cu(1)-N(1) | 1.9979(17) | Cu(1)-O(11) | 2.0024(13) |
| Cu(1)-N(3) | 2.0131(16) | Cu(1)-N(2) | 2.0387(16) |
| Cu(1)-N(4) | 2.2231(16) | | |
| | | | |
| N(1)-Cu(1)-O(11) | 90.64(6) | N(1)-Cu(1)-N(3) | 175.59(6) |
| O(11)-Cu(1)-N(3) | 89.38(6) | N(1)-Cu(1)-N(2) | 81.81(6) |
| O(11)-Cu(1)-N(2) | 160.60(6) | N(3)-Cu(1)-N(2) | 96.80(6) |
| N(1)-Cu(1)-N(4) | 105.16(6) | O(11)-Cu(1)-N(4) | 102.36(6) |
| N(3)-Cu(1)-N(4) | 79.14(6) | N(2)-Cu(1)-N(4) | 96.87(6) |

***Table 4:*** Selected bond lengths [Å] and angles [°] for $[\{Cu(phen)_2\}_2(terph)](terph)13.5H_2O.2EtOH$ (**6**)

| | | | |
|---|---|---|---|
| Cu-N(1A) | 1.990(4) | Cu-N(2A) | 2.053(4) |
| Cu-O(1) | 1.993(3) | Cu-N(1B) | 2.188(4) |
| Cu-N(2B) | 2.002(4) | | |
| | | | |
| N(1A)-Cu-O(1) | 91.31(14) | N(2B)-Cu-N(2A) | 95.07(15) |
| N(1A)-Cu-N(2B) | 173.98(15) | N(1A)-Cu-N(1B) | 95.91(14) |
| O(1)-Cu-N(2B) | 93.61(13) | O(1)-Cu-N(1B) | 98.65(13) |
| N(1A)-Cu-N(2A) | 81.96(15) | N(2B)-Cu-N(1B) | 79.92(15) |
| O(1)-Cu-N(2A) | 153.67(13) | N(2A)-Cu-N(1B) | 107.33(14) |

**S-3. Cytotoxicity Assays.**

**Human colon cancer cell line, HT29** (passage 17 to 30, ATCC, USA), was grown in McCoys's 5a medium supplemented with 2mM L-glutamine and fetal bovine serum (FBS) 10% and containing 100U/ ml penicillin and 100µg / ml streptomycin at 37ºC in a humidified atmosphere with 5% $CO_2$

**Human Breast Cancer cell line, MCF-7** (passage 16 to 26, ATCC, USA) was grown in Eagle's minimum essential medium containing 2mM L-glutamine, 100U/ ml penicillin,100µg/ ml streptomycin, FBS (10%), 1mM sodium pyruvate and 1% (v/v) non essential amino acids at 37ºC in a humidified atmosphere with 5% $CO_2$

**Human prostate cancer cell line, DU145** (passage 12 to 29, ATCC, USA) was grown in Eagle's minimum essential medium containing 2mM L-glutamine, 100U/ ml penicillin, 100µg/ ml streptomycin, FBS (10%), 1mM sodium pyruvate and 1% (v/v) non essential amino acids at 37ºC in a humidified atmosphere with 5% $CO_2$.

All drugs were tested by MTT assay following 24-hour exposure of cells to each compound. Each value represents the mean $IC_{50}$ of three independent experiments +/- standard error.

**MTT Assay.** This method is based on the reduction of the tetrazolium salt, methylthiazolyldiphenyl-tetrazolium bromide (MTT) into a crystalline blue formazan product by the cellular oxidoreductases of viable cells.[5] The resultant formazan crystal formation is proportional to the number of viable cells. Cells were seeded at 4x $10^5$ cells/ml in 96-well plates and incubated at 37ºC in 5% $CO_2$ for 24 hours. Cells were treated with a four log range of concentration of the test compounds in triplicate from 0.1 to 500 µM or with a solvent control (0.5% DMSO) in complete medium. Following 24 h incubation, cells were incubated with 20 µl of MTT (5 mg/ml) in 0.1 M PBS, pH 7.4 at 37 $^0$C in a humid atmosphere with 5% $CO_2$ for 4 h. Media was then gently aspirated from test cultures and 100 µL of dimethyl sulphoxide (DMSO) was added to all wells. The plates were then shaken for 2 min and the absorbance was read at 550 nm in a Varioscan plate reader. The IC50 was defined as the concentration of test compound required to reduce the absorbance of the MTT-formazan crystals by 50%, indicating 50% cell deactivation.

## S-4. Competitive ethidium displacement and quenching experiments.

**Competitive Ethidium Displacement.**[6] A working solution containing 1 μ*M* CT-DNA ($\varepsilon_{260}$ = 12,824 $M(bp)^{-1}$ $cm^{-1}$) along with 1.26 μ*M* ethidium bromide (EtBr) at neutral pH in TES buffer (10 m*M* TES, 0.1 m*M* $Na_2EDTA$, pH = 7.0) was prepared. Complexes **4-6**, actinomycin D, pentamidine isethionate, Cu(II) nitrate and OP were prepared at 2.0 m*M* in DMSO. Two millilitres of DNA-Et solution were placed in a 10-mm quartz cuvet (3 mL) and positioned in a temperature controlled ($20^oC$) spectrofluorometer (Perkin Elmer LS55B Luminescence Spectrometer). Excitation and emission wavelengths were set to 546 and 595 nm, respectively. After thermal equilibrium was established the emission and excitation slits were modified to give a fluorescence reading of 50 arbitrary units with measurements being recorded over a 20-s interval. An aliquot of complex or drug solution was taken (0.5 – 10 μL), was added to the cuvette and after equilibration the fluorescence reading was recorded. Repeated aliquots were added until the fluorescence was 20-40% of the initial control. Triplicate titrations were preformed and the apparent binding constants were calculated using $K_{app} = K_e \times 1.26/C_{50}$ where $K_e = 9.5 \times 10^6$ $M(bp)^{-1}$.

**DNA-Ethidium Fluorescence Quenching.**[6] Working solutions containing 20 μ*M* poly[d(A-T)]$_2$ ($\varepsilon_{260}$ = 13,100 $M(bp)^{-1}$ $cm^{-1}$) and poly[d(G-C)]$_2$ ($\varepsilon_{260}$ = 16,800 $M(bp)^{-1}$ $cm^{-1}$), along with 2.0 μ*M* Et in NaOAc buffer (2 m*M* NaOAc, 9.3 m*M* NaCl, 0.1 m*M* $Na_2EDTA$, pH = 5.0) were prepared. Subsequent experimental steps are identical to the displacement experiment with the exception that a fluorescence reading of 200 arbitrary units with measurements being recorded over a 20-s interval was used. From a plot of the fluorescence vs added drug concentration, the *Q* values is given by the concentration required to effect 50% removal of the initial fluorescence.

**S-5. Time-course experiments with DNA cleavage.**

**Nuclease Activity.** Reactions were carried out in a total volume of 20 μL in 0.1 *M* cacodylate buffer (pH = 6.0) with 1-100 μ*M* of complex, which were initially prepared in DMF, then diluted in buffer (in which they dissolved), with 1 μL of 0.25 μg/μL pUC18 (Roche). Samples were incubated for 5 hr at 37$^{o}$*C*. Quench buffer (3 μL; 0.25% bromophenolblue, 0.25% xylene cyanole and 30% glycerol) was then added and samples were loaded onto agarose gel (0.8 %) containing 1.5 μL/100 mL of solution of GelRed$^{TM}$ (10,000X). Electrophoresis was completed at 80 V for 2 hr in TAE buffer.

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