

## PAPER

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14364Unravelling the mechanism of apoptosis induced  
by copper(II) complexes of NN<sub>2</sub>-pincer ligands in  
lung cancer cells†Athulya Das and Muniyandi Sankaralingam \*

The invention of efficient chemotherapeutic drugs is essential for human health and development. Keeping this in mind, a series of copper(II) pincer complexes, **1–4**, of ligands **L1(H)** = 2-morpholino-*N*-(quinolin-8-yl)acetamide, **L2(H)** = 2-di-*n*-propylamino-*N*-(quinolin-8-yl)acetamide, **L3(H)** = 2-di-*n*-butylamino-*N*-(quinolin-8-yl)acetamide and **L4(H)** = 2-di-*n*-benzylamino-*N*-(quinolin-8-yl)acetamide have been synthesized, characterized, and utilized for inhibiting cancer proliferation. Complexes **1–4** showed very efficient activity against lung (A549) and breast (MCF-7) cancer cells, which are the most frequently diagnosed cancers according to the WHO. Among them, **1** was highly active against lung cancer cells with an IC<sub>50</sub> value of 8 μM, showing no toxicity towards common L929 fibroblast cell lines (IC<sub>50</sub> > 1000 μM). Moreover, AO–EB staining inferred that this cellular demise was attributed to apoptosis, which was determined to be 25.91% of cells by flow cytometry at the IC<sub>50</sub> concentration. Furthermore, carboxy-H<sub>2</sub>DCFDA staining revealed the involvement of ROS in the mechanism. Interestingly, JC-1 dye staining revealed a change in the potential of the mitochondrial membrane, which indicates the enhanced production of ROS in mitochondria. A deep search for the mechanism through *in silico* studies guided us to the fact that complexes **1–4** might perturb the function of complex I in mitochondria. Furthermore, the studies can be expanded towards clinical applications mainly with morpholine appended complex **1**.

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## 1. Introduction

According to the World Health Organization (WHO), cancer caused one among six deaths in 2018.<sup>1</sup> Even though the survival rate is improving, there is a long way to go for a complete cure of the disease. Lung cancer and breast cancer have become very common but an ideal treatment method is yet to be established. Therefore, scientists are on a huge thrust to develop novel, efficient drugs against this malady. As a result, recent years have witnessed the rapid expansion of metallo-drugs with a wide variety of metals including transition metals.<sup>2–7</sup> The choice of a metal centre plays an important role

because it can change the physicochemical properties of the complexes.<sup>8,9</sup> Moreover, biomimetic first-row transition metal complexes are found to be very promising candidates as anti-cancer drugs.<sup>10</sup> Among them, copper complexes always have a special place due to their high anticancer activity.<sup>11–14</sup> One of the relevant components of metallodrugs is the heterocyclic ligands that are extremely biocompatible due to the structurally relevant natural molecules.<sup>15–17</sup> For instance, aminoquinoline is one of the pharmacophores in medicinal chemistry involved in many applications including anticancer activities.<sup>18–20</sup> Similarly, along with aminoquinoline, if bio-active molecules like morpholine are used, then the activity could be enhanced.<sup>21</sup> Moreover, the solubility and lipophilicity can be tuned by propyl, butyl, and benzyl groups on the ligands.<sup>22</sup>

In the past few years, several pincer complexes of different metals have been reported to have excellent anticancer activities that can be attributed to their unique structural orientation.<sup>23</sup> For example, two of the aminoquinoline-based copper(II) pincer complexes were reported to have cytotoxic activity against A549 cells. Among them, one of the complexes had an IC<sub>50</sub> value of 1 μM and the other was active only at a concentration as high as 1 mM.<sup>24</sup> In another report, two more aminoquinoline based copper(II) pincer complexes were

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† Electronic supplementary information (ESI) available: Detailed procedure for synthesis and lipophilicity determination. Table S1 and Fig. S1–S16 include IR spectra of **L1(H)**–**L4(H)** and **1–4**, the mass spectrum of **4**, phase images, and percentage viability diagrams of A549 and MCF-7 treated with complexes and ligand **L1(H)**, phase images and percentage viability diagrams of L929 treated with **1**, UV-visible spectra of **L4(H)** and **4** for lipophilicity calculation, UV-visible spectra for complex **4** formation constant calculation, and molecular docking interactions of **2–4** with 5XTD. CCDC 2311348. For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d4dt01075b>

reported with  $IC_{50}$  values of around 0.46 and 0.43–0.90  $\mu\text{M}$ , respectively, against MCF-7 and A549 cell lines.<sup>25</sup> Another report described a series of terpyridine based copper(II) pincer complexes with good activity against A549 ( $IC_{50}$  = 4.2–100  $\mu\text{M}$ ) and MCF-7 ( $IC_{50}$  = 43–100  $\mu\text{M}$ ) cell lines.<sup>26</sup> Similarly, cyclometalated copper(I) pincer complexes with phosphadamantane derivatives were reported with very good anticancer activity against A549 and MCF-7 cells, for which the  $IC_{50}$  value ranges from 2.35 to 3.92  $\mu\text{M}$  and 3.08 to 8.41  $\mu\text{M}$ , respectively.<sup>27</sup> Very recently, SNS donor copper(II) pincer complexes have been reported against A549 cells.<sup>28</sup> Among them, the chloro and thiocyanate derivatives were more active with  $IC_{50}$  values of around 12.30  $\mu\text{M}$ . However, the perchlorate derivative had an  $IC_{50}$  value of 14.08  $\mu\text{M}$ . Thus, activity mainly changes depending on the ligand donors.

As previously mentioned, morpholine (1), dipropyl (2), dibutyl (3), and dibenzyl (4) derivatives of 8-aminoquinoline based copper(II) pincer complexes (Scheme 1) were synthesized, characterized, and tested for their anticancer efficacy against MCF-7 and A549 cell lines. It should be noted that complexes 1–3 have already been proved as better antimicrobial agents from our recent study.<sup>7</sup> Here, with  $IC_{50}$  values ranging from 8 to 20  $\mu\text{M}$  and 15 to 19.35  $\mu\text{M}$ , all four complexes showed potential cytotoxicity against A549 and MCF-7 cells, respectively. The highly active complex 1 against A549 cells was found to display no toxicity towards the normal L929 fibroblast cell line ( $IC_{50}$  > 1000  $\mu\text{M}$ ). The dominant activity of 1 compared to its corresponding ligand L1(H) ( $IC_{50}$  = 77.65  $\mu\text{M}$ ) against A549 cells revealed the importance of the copper complexes. A detailed mechanistic investigations reveal that cell death through apoptosis, which was induced by the present copper complexes, produces excess ROS in mitochondria. Furthermore, we expect that clinical applications with morpholine appended complex 1 may be extended.<sup>29</sup>

## 2. Experimental

### 2.1. Materials

Chemicals purchased commercially were used without additional purification. Bromoacetyl bromide and 8-aminoquinoline were obtained from TCI Chemicals. Copper(II) bromide, triethylamine, morpholine, and diethyl ether were procured

from Merck, India. Alfa Aesar supplied di-*n*-propylamine and di-*n*-butylamine. Dibenzylamine and DMSO (extra pure 99%) were procured from SRL, India. Acetone, methanol, dichloromethane, and ethyl acetate were procured from Qualigens, India. Using magnesium turnings and iodine, methanol was distilled. Calcium chloride and calcium hydride were used to distill acetone and DCM respectively. The cell lines A549 and MCF-7 were obtained from the National Centre for Cell Science (NCCS), Pune, India.

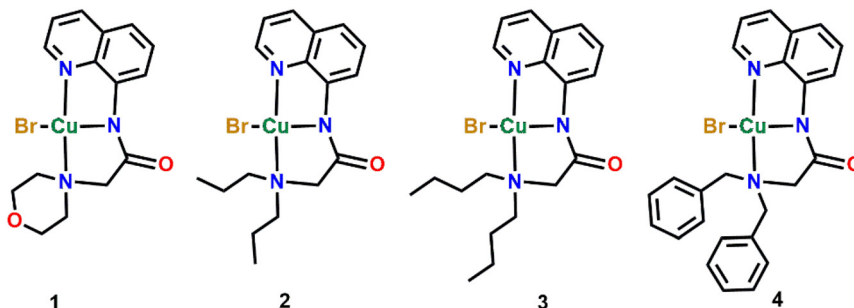
### 2.2. Physical measurements

Agilent Cary 8454 UV-visible and Jasco 4700 ATR-FTIR spectrometers were utilized for recording the absorption spectra and infrared spectra of the compounds, respectively. NMR spectra were recorded using a Jeol 500 MHz NMR spectrometer. Mass data were measured using an Agilent 6545 Q-TOF LC/MS instrument and a Bruker Daltonics Esquire 6000. A PerkinElmer 2400 Series II analyser was used for CHN analysis. A Bruker BioSpin spectrometer, Germany (EMXmicro A200-9.5/12/S/W), was used to collect electron paramagnetic resonance (EPR) spectra at 100 K. Calibration of the *g* value was done using a manganese marker. Magnetic measurements *viz.* zero-field cooling (ZFC) and field cooling (FC) were performed using Bruker WIN EPR acquisition and processing software in the range of 100–300 K. A Bruker D8 Venture dual source diffractometer was employed to obtain single-crystal X-ray diffraction data using Mo-K $\alpha$  radiation. An Olympus fluorescence microscope, CKX-53, Japan, was used to see the cells after anticancer mechanistic studies. All the incubations for anticancer studies were under a 5% CO<sub>2</sub> atmosphere at 37 °C.

### 2.3. Synthesis of ligands and complexes

The ligands L1(H), L2(H), and L3(H) and their copper(II) complexes 1, 2, and 3 were synthesized by following our very recent reports.<sup>6,7</sup> The detailed synthetic steps are elaborated in the ESI.<sup>†</sup>

**2.3.1. 2-Morpholino-*N*-(quinolin-8-yl)acetamide, L1(H).** Beige colour powder; yield, 79(3)%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 298 K);  $\delta$  (ppm): 11.38 (s, 1H), 8.78–8.77 (dd, *J* = 4.58, 1.53 Hz, 1H), 8.69–8.67 (dd, *J* = 7.25, 1.53 Hz, 1H), 8.08–8.06 (dd, *J* = 8.39, 2.29 Hz, 1H), 7.48–7.42 (m, 2H), 7.39–7.36 (dd, *J* = 8.01, 4.58 Hz, 1H), 3.82 (t, *J* = 4.58 Hz, 4H), 3.21 (s, 2H), 2.62 (t, *J* =



**Scheme 1** Copper(II) complexes of aminoquinoline based NN<sub>2</sub> pincer ligands.



4.58 Hz, 4H). ATR,  $\text{cm}^{-1}$  (Fig. S1†): 1694  $\text{cm}^{-1}$  (C=O) and 3265  $\text{cm}^{-1}$  (N-H).

**2.3.2. 2-Di-*n*-propylamino-*N*-(quinolin-8-yl)acetamide, L2 (H).** Off-white oil; yield, 90(2)%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  (ppm) 11.47 (s, 1H), 8.74–8.72 (m, 2H), 8.06–8.04 (dd,  $J = 8.39$ , 1.53 Hz, 1H), 7.47–7.41 (m, 2H), 7.36–7.34 (dd,  $J = 8.01$ , 4.58 Hz, 1H), 3.23 (s, 2H), 2.51 (t,  $J = 6.87$  Hz, 4H), 1.57–1.49 (m, 4H), 0.89 (t,  $J = 6.87$  Hz, 6H). ATR,  $\text{cm}^{-1}$  (Fig. S1†): 1676  $\text{cm}^{-1}$  (C=O) and 3264  $\text{cm}^{-1}$  (N-H).

**2.3.3. 2-Di-*n*-butylamino-*N*-(quinolin-8-yl)acetamide, L3 (H).** Yellow oil; yield, 80(2)%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  (ppm) 11.54 (s, 1H), 8.83–8.80 (m, 2H), 8.15–8.13 (dd,  $J = 8.39$ , 1.53 Hz, 1H), 7.55–7.49 (m, 2H), 7.45–7.42 (dd,  $J = 8.39$ , 4.58 Hz, 1H), 3.30 (s, 2H), 2.61 (t,  $J = 6.87$  Hz, 4H), 1.60–1.54 (m, 4H), 1.46–1.38 (m, 4H), 0.89 (t,  $J = 6.87$  Hz, 6H). ATR,  $\text{cm}^{-1}$  (Fig. S1†): 1680  $\text{cm}^{-1}$  (C=O) and 3245  $\text{cm}^{-1}$  (N-H).

**2.3.4. 2-Di-*n*-benzylamino-*N*-(quinolin-8-yl)acetamide, L4 (H).** Ligand L4(H) was synthesized using a previously reported procedure with slight modifications.<sup>6,7</sup> 2-Bromo-*N*-(quinolin-8-yl)acetamide (1 g,  $3.77 \times 10^{-3}$  mol) and three equivalents of dibenzylamine (2.23 g,  $11.3 \times 10^{-3}$  mol) were dissolved in acetone and refluxed overnight. The reaction mixture was cooled and subjected to extraction using ethyl acetate followed by brine solution. The organic fraction was dried over sodium sulfate and concentrated under reduced pressure. A silica column was used with a hexane (4): ethyl acetate (1) mixture to obtain the pure ligand with a yield of 60(2)% as a white powder.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 298 K)  $\delta$  (ppm): 11.67 (s, 1H), 9.03 (s, 1H), 8.76–8.73 (m, 1H), 8.20–8.18 (dd,  $J = 8.20$ , 1.53 Hz, 1H), 7.67–7.65 (d,  $J = 6.87$  Hz, 4H), 7.54–7.52 (m, 3H), 7.33–7.30 (t,  $J = 7.25$  Hz, 4H), 7.25–7.22 (m, 2H), 3.76 (s, 4H), 3.38 (s, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ , 298 K)  $\delta$  (ppm): 58.89, 59.09, 116.40, 121.59, 121.67, 127.34, 127.41, 128.07, 128.47, 129.22, 134.47, 136.21, 138.17, 138.79, 148.19, 169.99. ATR,  $\text{cm}^{-1}$  (Fig. S1†): 1676  $\text{cm}^{-1}$  (C=O) and 3280  $\text{cm}^{-1}$  (N-H).

**2.3.5. [Cu(L1)(Br)], 1.** Green colour; yield, 75(1)%. Single crystals were obtained by the slow evaporation of a methanol–chloroform solution of 1 in two days. Elemental analysis calcd. for ( $\text{C}_{15}\text{H}_{18}\text{N}_3\text{O}_3\text{BrCu}$ ): C 41.82, H 3.98, N 9.75; found: C 41.79, H 3.97, N 9.72.

**2.3.6. [Cu(L2)(Br)], 2.** Green colour; yield, 65(2)%. The crystalline compounds were obtained in methanol solvent. Elemental analysis calcd. for ( $\text{C}_{17}\text{H}_{22}\text{N}_3\text{OBrCu}$ ): C 47.73, H 5.18, N 9.82; found: C 47.71, H 5.16, N 9.79.

**2.3.7. [Cu(L3)(Br)], 3.** Green colour; yield, 66(2)%. The crystalline compounds were obtained in methanol solvent. Elemental analysis calcd. for ( $\text{C}_{19}\text{H}_{26}\text{N}_3\text{OBrCu}$ ): C 50.06, H 5.75, N 9.22; found: C 49.96, H 5.73, N 9.19.

**2.3.8. [Cu(L4)(Br)], 4.** Copper(II) bromide (0.1 g,  $4.47 \times 10^{-4}$  mol) was dissolved in methanol. Then ligand L4(H) (0.17 g,  $4.47 \times 10^{-4}$  mol) was added to the methanolic solution of copper(II) bromide under constant stirring. Following this, triethylamine (TEA, 0.062 mL,  $4.47 \times 10^{-4}$  mol) was added to the reaction mixture to remove the amide N–H proton. The reaction continued for 4 h and isolated the green coloured precipitate with a yield of 53(2)%. ATR-IR data ( $\text{cm}^{-1}$ ) (Fig. S2†):

1597  $\text{cm}^{-1}$  (C=O), QTOF MS  $m/z$  (%) (Fig. S3†): 443.10 (calcd), 443.10 [ $\text{Cu(L4)}^+$ ] (found). Elemental analysis calcd. for ( $\text{C}_{25}\text{H}_{22}\text{N}_3\text{OBrCu}$ ): C 57.31, H 4.23, N 8.02; found: C 57.22, H 4.21, N 7.99.

## 2.4. Single-crystal X-ray diffraction studies

By careful examination under an optical microscope, fine single crystals suitable for X-ray diffraction were chosen from the mother liquor of complex 1. Single crystals were mounted using a fibre loop and optically centered. The automatic cell determination routine was employed to collect reflections (24 frames at three different orientations of the detector) and the APEX3-SAINT program was used for determining the unit cell parameters.<sup>30</sup>

The data were corrected for Lorentz-polarization effects. Semiempirical absorption correction (multi-scan) based on symmetry-equivalent reflections was applied using the SADABS program.<sup>31</sup> The structures were solved by direct methods and refined by full-matrix least-squares, based on  $F^2$  using SHELX-2018 software package<sup>2</sup> and the WinGX program.<sup>32</sup> The crystallographic details of the data collected for 1 are given in Table 1.

## 2.5. MTT assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay can discern the metabolic activity using the colorimetric technique. In the presence of viable cells, MTT turns into purple formazan from yellow colour.<sup>33,34</sup> Once the cell culture was trypsinized, a medium containing 10% fetal bovine serum (FBS) was used to adjust the cell count to  $1 \times 10^5$  cells per mL. Then, they were filled in 96-well microliter plates

**Table 1** Crystallographic data for complex 1

	1
Empirical formula	$\text{C}_{15}\text{H}_{18}\text{BrCuN}_3\text{O}_3$
Formula weight, $\text{g mol}^{-1}$	431.77
Crystal habit, colour	Block, green
Crystal system	Monoclinic
Crystal size, mm	$0.042 \times 0.030 \times 0.025$
Space group	$P2_1/n$
$a$ , Å	7.9149(8)
$b$ , Å	18.466(2)
$c$ , Å	11.1514(11)
$\alpha$ , °	90
$\beta$ , °	94.607(7)
$\gamma$ , °	90
$V$ (Å <sup>3</sup> )	1624.6(3)
$Z$	4
$\rho_{\text{calcd}}/\text{mg m}^{-3}$	1.765
$F(000)$	868
Temperature (K)	298(2)
No. of reflections collected	9012
Independent reflections	2055 [ $R(\text{int}) = 0.1366$ ]
Radiation (Mo- $\text{K}\alpha$ )/Å	1.54178
Goodness-of-fit on $F^2$	1.203
Number of refined parameters	214
$R_1/wR_2$ [ $I > 2\sigma(I)$ ] <sup>a</sup>	$R_1 = 0.1300$ , $wR_2 = 0.3196$
$R_1/wR_2$ (all data)	$R_1 = 0.2503$ , $wR_2 = 0.4030$

$$^a R_1 = [\sum(|F_o| - |F_c|)/\sum|F_o|]; wR_2 = \{[\sum(w(F_o^2 - F_c^2)^2)/\sum(wF_o^4)]^{1/2}.$$



in such a way that each well contained 50000 cells. A partial monolayer was formed after 24 h. This monolayer was rinsed with the medium and compounds dissolved in DMSO were added in serial dilutions from 20  $\mu\text{M}$  to 0  $\mu\text{M}$ . Furthermore, it was incubated for 24 h. Then, 100  $\mu\text{L}$  of 5 mg per 10 mL MTT in PBS (phosphate buffered saline) was distributed in each well and incubation was continued for 4 h. Following the removal of the supernatant, 100  $\mu\text{L}$  of DMSO was introduced to solubilize the formazan. The absorbance of this solution was determined using a microplate reader (Tecan plate reader) at 590 nm. The following formula was used to determine the extent of cell growth inhibition:

$$\% \text{Inhibition} = 1 - \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100 \quad (1)$$

Furthermore, the dose–response curve between different concentrations of compounds against the percentage inhibition was able to obtain the half-maximal inhibitory concentration ( $\text{IC}_{50}$ ). The  $\text{IC}_{50}$  was computed using GraphPad Prism 6 (GraphPad, San Diego, CA, USA). The  $\text{IC}_{50}$  values are calculated based on the mean of the triplicate absorptions. In addition, mechanistic studies were carried out for complex **1** against A549 cells due to its elevated activity compared to other complexes.

## 2.6. Acridine orange (AO)–ethidium bromide (EB) staining

Cells treated with **1** were examined for apoptosis using a dual AO–EB fluorescence staining method.<sup>35–37</sup> In a nutshell, 4000 cells per well in 24 well plates were processed with **1** at the  $\text{IC}_{50}$  concentration and incubated for 24 h. The staining solution containing AO and EB at a concentration of 100  $\text{g mL}^{-1}$  was added to each well at a volume of 500  $\mu\text{L}$  after 24 h of incubation. The results were observed through a fluorescence microscope.

## 2.7. Flow cytometry

Annexin V-FITC/PI staining is considered as a sophisticated technique that can distinguish cells of viable, apoptotic, and necrotic features.<sup>38–40</sup> Once  $10^5$  cells per well were cultured under incubation for 24 h in a 6-well plate, they were treated with **1** at the  $\text{IC}_{50}$  concentration. The cells were trypsinized, and after 24 h of incubation, they were rinsed with PBS and dyed with Annexin V-FITC/PI in accordance with the instructions on the Annexin V-FITC apoptosis detection kit. The cells that have not undergone treatment with **1** were treated as the control. Furthermore, the extent of cell death was evaluated using a SYSMEX flow cytometer (Japan) and analyzed using FlowJo software.

## 2.8. Determination of ROS

Carboxy- $\text{H}_2\text{DCFDA}$  (2',7'-dichlorodihydrofluorescein diacetate) is normally non-fluorescent. Once ROS is present, it oxidises to show green fluorescence.<sup>41–43</sup> A fresh stock solution of carboxy- $\text{H}_2\text{DCFDA}$  was prepared in sterile 100% ethanol right before use. After 24 hours, **1** at the  $\text{IC}_{50}$  concentration was treated with carboxy- $\text{H}_2\text{DCFDA}$  (1  $\mu\text{M}$ ) in a regular culture

medium with reduced serum (2%) and incubated for 30 minutes. The medium containing carboxy- $\text{H}_2\text{DCFDA}$  was removed and washed with PBS twice. A fluorescence microscope was used to examine the cells.

## 2.9. Determining the mitochondrial membrane potential (MMP) by JC-1 staining

Roughly  $5 \times 10^5$  cells per mL were cultured on coverslips in a 6-well culture plate and incubated for 8 h.<sup>44–46</sup> After 24 h of incubation with **1** at the  $\text{IC}_{50}$  concentration, 1–10  $\mu\text{M}$  of JC-1 dye was added to the cell culture medium as a working stock solution and the plates were incubated (15–30 min). The dead cell percentage was immediately observed under a fluorescence microscope.

## 2.10 Lipophilicity and Stability studies

The lipophilicity was determined by the shake-flask method using a pre-saturated 1-octanol–water solution which is detailed in the ESI.<sup>†</sup><sup>47</sup> The stability of the complexes was calculated according to their decay in DMSO and FBS using UV-vis spectroscopy. The absorption spectra of compounds dissolved in DMSO and 5% DMSO in FBS were recorded at 0 h and after 24 h. The percentage of decay was calculated from the differences in the absorption of both spectra.

## 2.11. Molecular docking

The interaction of complexes **1–4** with complex I of mitochondria was performed using AutoDock Vina.<sup>48</sup> The protein structure, 5XTD, was obtained from the Protein Data Bank. The active site was included completely by creating grid box dimensions of  $46 \text{ \AA} \times 66 \text{ \AA} \times 74 \text{ \AA}$  and the same was used for all the complexes. The Gaussian 09 software package at the B3LYP level of theory and the basis sets 6-31G for C, H, O, and N and LANL2DZ for the metal centre were used to optimize the geometry of the complexes.<sup>49</sup> The output was visualized and analysed using Autodock tools 1.5.7 and Discovery Studio.<sup>50</sup>

# 3. Results and discussion

## 3.1. Synthesis and characterization of ligands and copper(II) complexes

As per the literature method, ligands **L1(H)**, **L2(H)**, **L3(H)** and **L4(H)** were obtained by reacting 2-bromo-*N*-(quinoline-8-yl) acetamide with morpholine, diisopropylamine, dibutylamine, and dibenzylamine, respectively.<sup>6,7</sup> Then, by stirring a methanolic solution of an equivalent amount of ligand,  $\text{CuBr}_2$ , and triethylamine, copper(II) complexes **1–4** were synthesized as reported.<sup>7</sup> The isolated compounds were characterized using advanced analytical techniques. For instance, the removal of a N–H peak during complex formation was clearly visible from the disappearance of the peak around  $3245\text{--}3280 \text{ cm}^{-1}$  in the ATR-IR spectra of **4** compared to the corresponding ligand **L4(H)**. This observation was similar for **1–3** and **L1(H)–L3(H)**.<sup>7</sup> Mass spectrometry also revealed the formation of **4**. A square planar coordination geometry of the copper(II) centre of **1–4** in



solution was indicated by a highly intense peak around 356–364 nm and a peak corresponding to a d–d transition around 631–655 nm in the UV-visible spectra. Complex **4** was observed with  $g$ -tensors ( $g_{\parallel} = 2.293$  and  $g_{\perp} = 2.039$ ) and coupling constants ( $A_{\parallel} = 194 \times 10^{-4} \text{ cm}^{-1}$ ) gained from the axial symmetry EPR spectra that match very well with the recently reported EPR spectra of **1–3**.<sup>7</sup> Furthermore, structural characterization of complex **1** by single-crystal X-ray crystallography revealed a distorted square pyramidal geometry that can be formulated as  $[\text{Cu}(\text{L1})\text{Br}(\text{H}_2\text{O})]$ .

### 3.2. Infrared spectra of **1–4** with **L1(H)–L4(H)**

Ligands **L1(H)–L4(H)** exhibited two major peaks around 3245–3280 and 1676–1694  $\text{cm}^{-1}$  in the functional group region, which are assigned to N–H (**L1(H)**, 3265  $\text{cm}^{-1}$ ; **L2(H)**, 3264  $\text{cm}^{-1}$ ; **L3(H)**, 3245  $\text{cm}^{-1}$ ; and **L4(H)**, 3280  $\text{cm}^{-1}$ ) and C=O (**L1(H)**, 1694  $\text{cm}^{-1}$ ; **L2(H)**, 1676  $\text{cm}^{-1}$ ; **L3(H)**, 1680  $\text{cm}^{-1}$ ; and **L4(H)**, 1676  $\text{cm}^{-1}$ ) groups. Upon complexation with  $\text{CuBr}_2$  in the presence of TEA, the N–H peak completely disappeared in **1–4**, revealing  $\text{N}^-$  (amido) coordination to the copper centre (Fig. S1 and S2†). Furthermore, the decrease in C=O stretching frequencies (1587–1597  $\text{cm}^{-1}$ ) was also noticed after complexation, indicating that the electron density localized more towards the copper centre. All these results were in concordance with the data recently reported by us.<sup>7</sup>

### 3.3. Electronic properties of complexes

Complexes **1–4** showed two peaks in the UV-visible spectra in methanol at room temperature. The observed highly intense peaks at around 356–364 nm (**1**, 356 nm ( $\epsilon$ , 4610  $\text{M}^{-1} \text{ cm}^{-1}$ ); **2**, 357 nm ( $\epsilon$ , 4095  $\text{M}^{-1} \text{ cm}^{-1}$ ); **3**, 357 nm ( $\epsilon$ , 4755  $\text{M}^{-1} \text{ cm}^{-1}$ ); and **4**, 364 nm ( $\epsilon$ , 4070  $\text{M}^{-1} \text{ cm}^{-1}$ )) are assigned to the charge transfer transition from the ligand to the copper(II) centre (LMCT) (Fig. 1). The other broad peaks at around 631–655 nm (**1**, 655 nm ( $\epsilon$ , 145  $\text{M}^{-1} \text{ cm}^{-1}$ ); **2**, 631 nm ( $\epsilon$ , 130  $\text{M}^{-1} \text{ cm}^{-1}$ ); **3**, 633 nm ( $\epsilon$ , 160  $\text{M}^{-1} \text{ cm}^{-1}$ ); and **4**, 647 nm ( $\epsilon$ , 135  $\text{M}^{-1} \text{ cm}^{-1}$ )) with a low intensity are assigned to the d–d transition ( $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$  in square planar copper(II) complexes).<sup>7,51</sup>

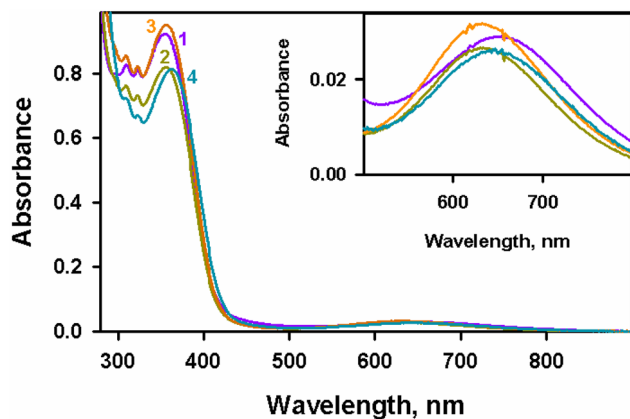


Fig. 1 UV-visible spectra of **1–4** ( $2 \times 10^{-4} \text{ M}$ ) recorded in methanol at 298 K.

The frozen EPR spectra of complex **4** exhibit a well-refined axial symmetry (Fig. 2), with  $g$ -tensors  $g_{\parallel} = 2.293$  and  $g_{\perp} = 2.039$  and coupling constants ( $A_{\parallel} = 194 \times 10^{-4} \text{ cm}^{-1}$ ), which were comparable to those reported for complexes **1–3** ( $g_{\parallel} = 1$ , 2.285; **2**, 2.283; and **3**, 2.274 and  $g_{\perp} = 1$ , 2.042; **2**, 2.039; and **3**, 2.033) and coupling constants ( $A_{\parallel}$  in  $\times 10^{-4} \text{ cm}^{-1} = 1$ , 197; **2**, 197; and **3**, 197).<sup>7</sup> In addition to low  $g_{\parallel}$  and high  $A_{\parallel}$  values, it is observed that the  $f$  quotient (118  $\text{cm}^{-1}$ ) falls within 105–135  $\text{cm}^{-1}$ , which suggests that the planar nature of complex **4** is similar to the other complexes **1–3**.<sup>7,52</sup> Moreover, the square-based geometry of the complexes was perceptible from the  $g_{\parallel} > g_{\perp}$  values, which indicates that  $d_{x^2-y^2}$  is the ground state.<sup>53</sup>

### 3.4. Molecular geometry of **1**

The molecular structure of complex **1** is shown in Fig. 3 with the atom numbering scheme, principal bond lengths and bond angles provided in Table 2. It was observed that **1** exhibits a distorted square pyramidal geometry with a monoclinic crystal system and the  $P2_1/n$  space group. The tridentate chelating ligand is coordinated in a facial fashion towards the copper(II) centre with the nitrogen atoms of quinolyl, amido and morpholine, along with a bromine atom (Br) and a water molecule (O(3)). This water molecule (O(3)) might be present in the solvent of crystallization, revealed by the lack of a peak corresponding to OH in the IR spectra of **1** (Fig. S2† (1)). Moreover, the coordination around the copper(II) of **1** with bond angles ranging from 91.7° to 110.4° that deviate from the ideal square pyramidal angles of 90° and 180° was observed. Here, the coordination around copper gives a trigonality index

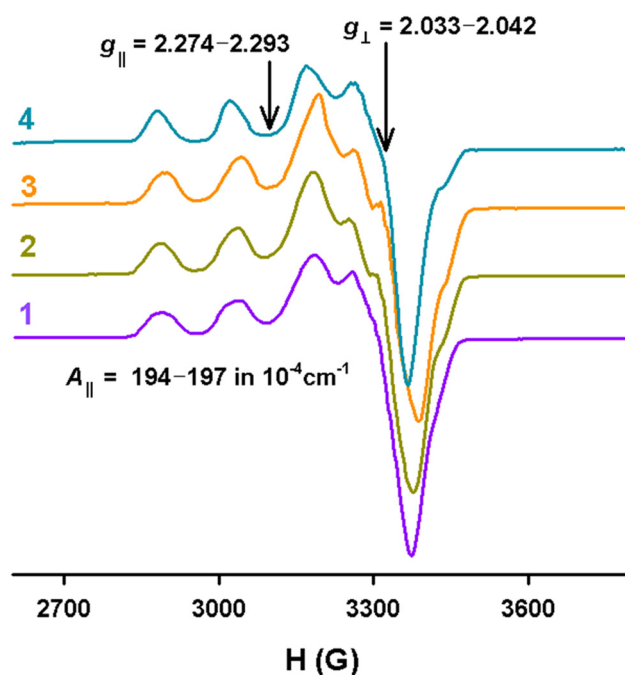
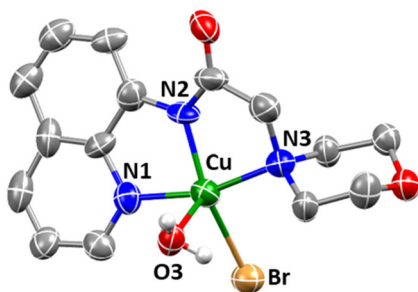


Fig. 2 A comparison of the frozen EPR spectrum of **4** with that of **1–3** in DMF : methanol (4 : 1 v/v) measured at 100 K.<sup>7</sup>





**Fig. 3** ORTEP diagram of **1** showing 30% probability of thermal ellipsoids, and the labelling scheme of the atoms. Hydrogen atoms in the chelating ligands are omitted for clarity.

**Table 2** Selected bond lengths [Å] and bond angles [°] of **1**

	Bond lengths/Å
Cu–N(1)	2.004(16)
Cu–N(2)	1.909(17)
Cu–N(3)	2.070(16)
Cu–Br	2.456(5)
Cu–O(3)	2.251(16)
	Bond angles [°]
N(1)–Cu–N(2)	80.2(9)
N(1)–Cu–N(3)	164.0(8)
N(2)–Cu–N(3)	83.8(8)
N(1)–Cu–Br	95.7(6)
N(2)–Cu–Br	152.0(6)
N(3)–Cu–Br	98.1(6)
N(1)–Cu–O(3)	91.7(6)
N(2)–Cu–O(3)	110.4(7)
N(3)–Cu–O(3)	94.5(6)
O(3)–Cu–Br	97.3(5)

( $\tau_5$ ) of 0.2 ( $\tau_5 = (\beta - \alpha)/60$ , where  $\alpha = \text{N}(2)\text{--Cu}(1)\text{--Br} = 152^\circ$  and  $\beta = \text{N}(1)\text{--Cu}(1)\text{--N}(3) = 164^\circ$ ).<sup>54</sup> As expected, the bond length of Cu(II)–N<sub>quinoline</sub> (N(1)) is shorter than the bond distance of Cu(II)–N<sub>morpholine</sub> (N(3)) due to the sp<sup>2</sup> hybridized quinoline ring.

Very recently, complexes **2** and **3** have been reported with a square planar geometry that is slightly distorted and crystallize with the space groups *C2/c* and *P2<sub>1</sub>/n*, respectively, in a monoclinic system.<sup>7</sup> Upon examining the bond parameters of **2** and **3**, it is found that the bond angles and bond lengths are comparable to those of complex **1**. It should be noted that in all complexes (**1–3**), the Cu–N<sub>amine</sub> (2.070(16)–2.0901(16) Å) bond is lengthier than the Cu–N<sub>quinoline</sub> (2.004(16)–2.0457(16) Å) bond, which is consistent with the sp<sup>3</sup> and sp<sup>2</sup> hybridizations, respectively. Moreover, these complexes deviate from their ideal structures with trigonality indexes of  $\tau_5 = 0.2$  for **1**,  $\tau_4 = 0.16$  for **2**, and 0.24 for **3**. Also, the distorted square pyramidal coordination of **1** was found to be similar to that of the recently reported zinc(II) complex [Zn(L1)Cl(H<sub>2</sub>O)] with a  $\tau_5$  value of 0.257.<sup>6</sup>

### 3.5. Activity of **1–4** against lung (A549) and breast (MCF-7) cancer cell lines

**3.5.1. The impact of **1–4** on cellular viability.** The effect of complexes **1–4** on A549 and MCF-7 cell lines was examined

from the diagrams of percentage viability (Fig. 4A and S4†), which clearly demonstrate the complex's enhanced activity with an increase in concentration. All the complexes exhibited a promising antiproliferative effect against A549 (IC<sub>50</sub> (μM): **1**, 8; **2**, 13.95; **3**, 13.85; and **4**, 20) (Table S1†) and MCF-7 (IC<sub>50</sub> (μM): **1**, 15; **2**, 17.55; **3**, 15.75; and **4**, 19.35) cancer cells (Fig. 4B). The most promising cytotoxic effect was shown by **1** against A549 cells (IC<sub>50</sub> = 8 μM). The activity of **1–4** is clearly visible from the culture morphology phase images provided in Fig. 5 and S5, S6.† Since the MTT assay was performed by dissolving compounds in DMSO and the biological medium of FBS, the stability of complexes was checked in both using UV-vis spectroscopy techniques. This shows that approximately 94–99.5% of complexes remained without any decay in DMSO (Fig. S7†) and 89–91% in FBS (Fig. S8†). It should be noted that in the case of **4** in FBS, the stability could not be calculated due to the formation of turbidity after 24 h (Fig. S8†). To understand more about the importance of this copper complex, the cytotoxicity of the corresponding ligand **L1(H)** was also examined (Fig. S9†). Almost a 10-fold increase in cytotoxicity is observed after the complexation of **L1(H)** (IC<sub>50</sub> (μM) = A549, 77.65; MCF-7, 77.52) with copper (Fig. S10†). The selectivity and excellent cytotoxicity of **1** against A549 cell lines made us proceed with further mechanistic studies. Moreover, the non-toxic nature of **1** against healthy cells was identified by treating it against a normal L929 fibroblast cell line (IC<sub>50</sub> > 1000 μM) (Fig. S11†). It is important to note that the IC<sub>50</sub> value of cisplatin against A549 cells is 29.25 μM under identical conditions, revealing that the present copper(II) complexes could be beneficial for overcoming the limitations of Pt-based chemotherapeutics.<sup>6</sup> It is also evident that **1–4** show good activity compared with the reported copper complexes of similar ligand architectures.<sup>25–29</sup> For example, the selected pincer-type copper complexes [Cu(MQA)(OAc)] where MQA = L-methionine-*N'*-8-quinolylamide, [CuCl<sub>2</sub>(thiophen-2-yl-terpy)] where terpy = 2,2':6',2''-terpyridine, [Cu(Me)(*N*-TP)(PF<sub>6</sub>)], [Cu(Et)(*N*-TP)(PF<sub>6</sub>)], and [Cu(*i*Pr)(*N*-TP)(PF<sub>6</sub>)] where *N*-TP = *N*-aryl-1,3,5-triaza-7-phosphaadamantane, [Cu(L)Cl](ClO<sub>4</sub>), and [Cu(L)Br<sub>2</sub>] where L = 2-((quinolin-8-ylimino)methyl)pyridine exhibit IC<sub>50</sub> (μM) values of 1, 4.2, 3.92, 3.14, 2.35, 0.90, and 0.43, respectively, against A549 cells.<sup>25–28</sup> It should be noted that, even though these complexes have lower IC<sub>50</sub> values than the present complex **1** (incubation time: 24 h), their incubation time is high (≥48 h).

Similarly, complexes [Cu(κ<sup>3</sup>-L)(Cl)<sub>2</sub>]·3H<sub>2</sub>O, [Cu(κ<sup>3</sup>-L)(SCN)<sub>2</sub>], and [Cu(κ<sup>3</sup>-L)(ClO<sub>4</sub>)<sub>2</sub>], where L = 2,6-bis[[4-bromophenyl]thio]methylpyridine, show results (IC<sub>50</sub> = 12.38–14.98 μM) comparable to the present complexes against A549 cells under incubation for 48 h.<sup>29</sup> Other complexes [Cu(CMQA)(H<sub>2</sub>O)] where CMQA = *N*-carboxymethyl-L-methionine-*N'*-8-quinolylamide, [CuCl<sub>2</sub>(1-methyl-1*H*-pyrrol-2-yl-terpy)], and [CuCl<sub>2</sub>(R-dtpy)] where R = furan-2-yl, thiophen-2-yl, 1-methyl-1*H*-pyrrol-2-yl and dtpy = 2,6-di(thiazol-2-yl)pyridine show less activity against A549 cells (IC<sub>50</sub> = 64.3–100 μM) compared to the present complexes **1–4**.<sup>25,27</sup>

In a similar way, comparing the reported activity of copper pincer complexes against MCF-7 revealed that [Cu(Me)(*N*-TP)(PF<sub>6</sub>)], [Cu(Et)(*N*-TP)(PF<sub>6</sub>)], [Cu(*i*Pr)(*N*-TP)(PF<sub>6</sub>)], [Cu(L)Cl](ClO<sub>4</sub>),



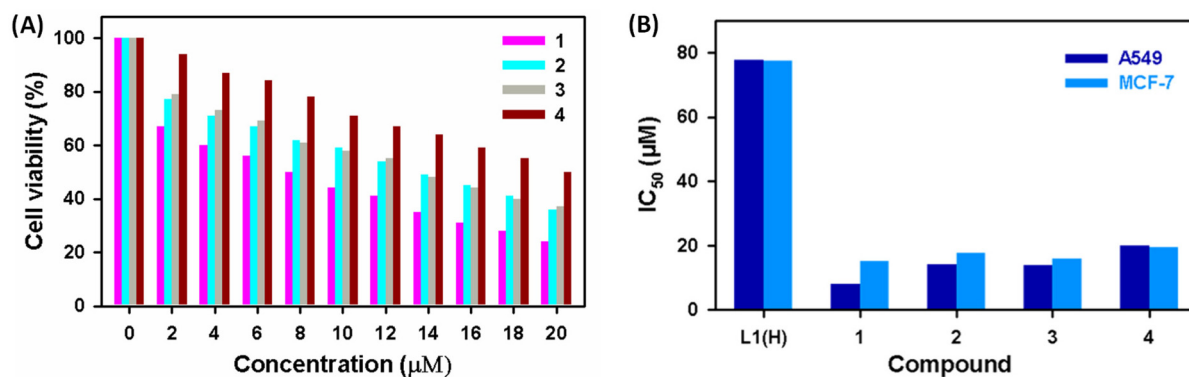


Fig. 4 (A) Viability of A549 under complex 1 and (B) IC<sub>50</sub> values of ligand L1(H) and complexes 1–4 effective against A549 and MCF-7 cancer cell lines.

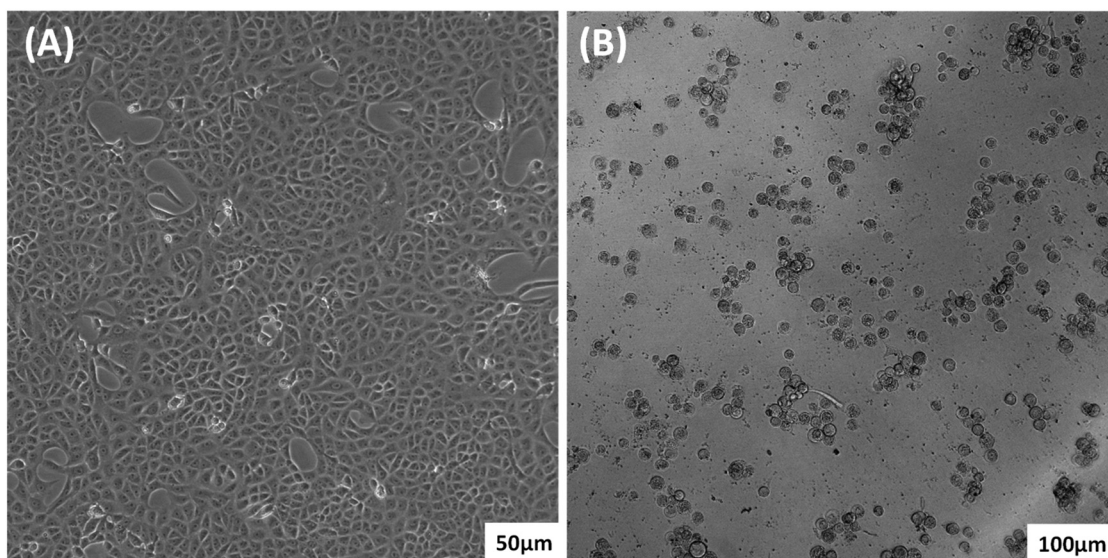


Fig. 5 Phase images of (A) control A549 cells and (B) A549 cells treated with 1.

and [Cu(L)Br<sub>2</sub>] have IC<sub>50</sub> values in between 0.46 and 8.41 μM, at an incubation time of 48 h. At the same time, it is observed that complexes [CuCl<sub>2</sub>(R-terpy)] and [CuCl<sub>2</sub>(R-dtpy)] where R = furan-2-yl, thiophen-2-yl, and 1-methyl-1H-pyrrol-2-yl are less active than 1–4 with IC<sub>50</sub> values ranging between 43 and 100 μM against MCF-7 at 48 h of incubation.<sup>27</sup> Very recently, we have reported the anticancer activity of zinc complexes of ligands L1(H)–L3(H) against A549 and MCF-7 cells and found that the IC<sub>50</sub> values range from 16.35 to 17.95 and 33.35 to 40 μM, respectively.<sup>6</sup> This diminished activity of zinc analogues indicates the supremacy of copper complexes in cancer therapy. Moreover, among the copper(II) complexes, 1 of L1(H) has the highest activity but in the case of zinc(II) complexes, 3 of L3(H) has the best activity. From this, it is understood that the ligand system also affects the activity along with the choice of metal ions.

**3.5.2. Complex 1 induced apoptosis in A549 cells: detection and quantification.** The cytological changes that occurred in A549 cells treated with 1 using the AO–EB staining assay were

examined.<sup>28</sup> As shown in Fig. 6A, the viable cells are observed as well-organized structures having nuclei with green fluorescence. Other morphological changes were observed after treating the cells with 1 as shown in Fig. 6B. Here, vivid green patches of perinuclear chromatin, red fluorescent nuclei, and swollen red luminous nuclei without chromatin rupture are an indication of early apoptotic, late apoptotic, and necrotic cells, respectively.

Furthermore, the quantification of apoptosis in A549 cells induced by 1 was marked using flow cytometry. A549 cells treated with 1, at the IC<sub>50</sub> concentration, showed a different extent of cell death compared with the control, as shown in Fig. 7. This resulted in 2.61% (Q3) of cells under apoptosis, 23.3% (Q2) of cells in the late apoptotic stage, and 5.47% (Q1) of necrotic cells (Fig. 7). The impact of 1 is clearly visible by comparing the results with that of the control (Q2 = 0.076%, Q3 = 0.41%, and Q1 = 3.1%). These findings altogether revealed that apoptosis is the primary mechanism by which the complex caused cell death. Similarly, the reported terpyri-



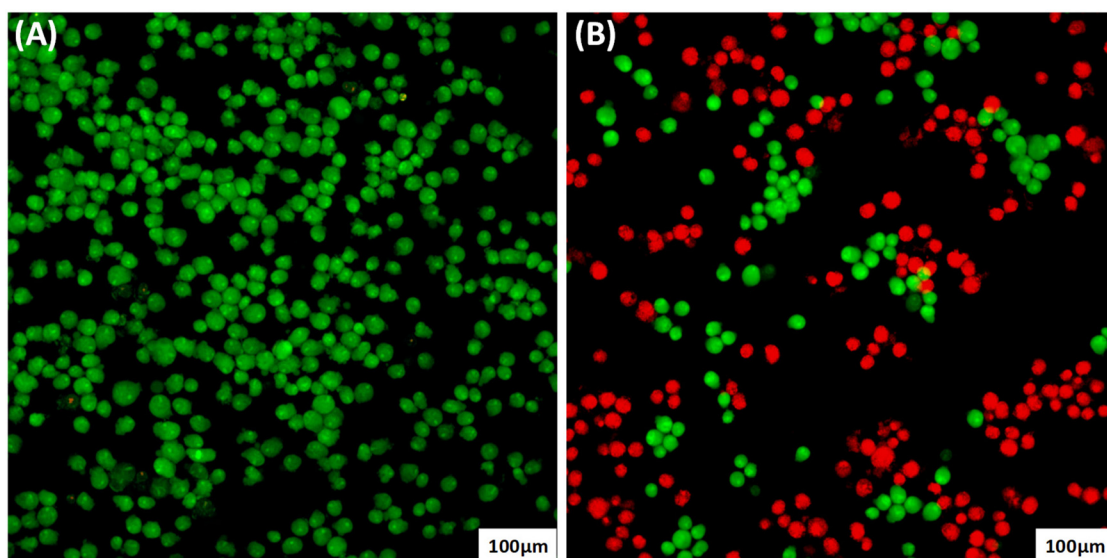


Fig. 6 AO-EB stained (A) A549 cells and (B) A549 cells treated with **1** at the IC<sub>50</sub> concentration.

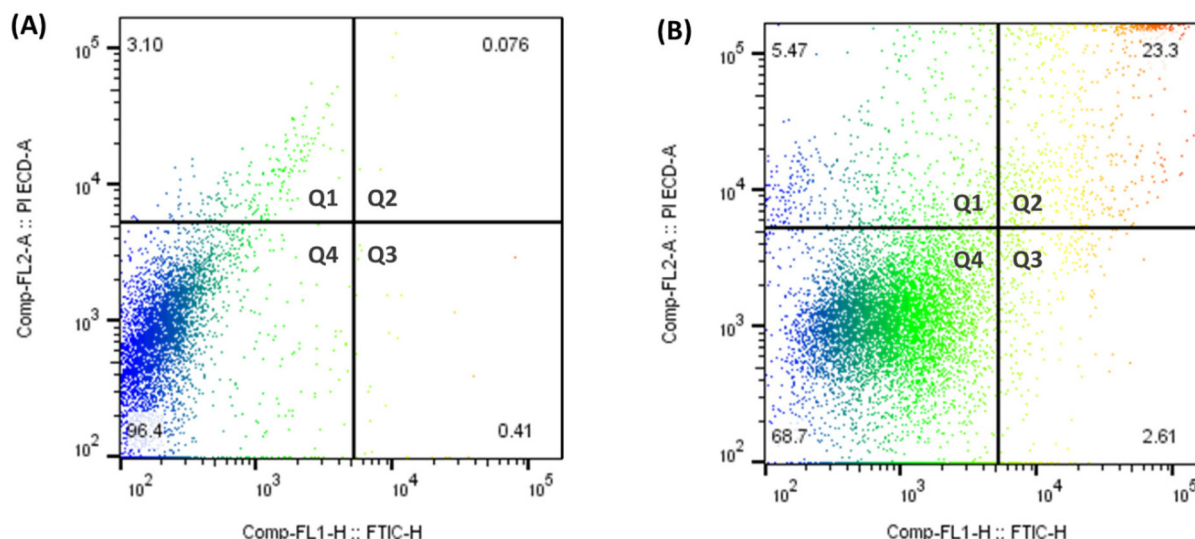


Fig. 7 Annexin V-FITC/PI staining in A549 cells: (A) control and (B) after being treated with **1** at the IC<sub>50</sub> concentration.

dine based copper(II) pincer complexes were active against A549 and MCF-7 cell lines and were able to induce apoptosis in A549 cells.<sup>27</sup> Similarly, the reported zinc(II) complex of **L3** (**H**) had a slightly low percentage of cells under apoptotic (1.37%), late apoptotic (21.4%), and necrotic (4.94%) cells compared to that of the present copper(II) complexes in the flow cytometry experiment.<sup>6</sup>

**3.5.3. Root cause of apoptosis in A549 cells by **1**.** The primary mechanism of anticancer activity by copper complexes involves excessive ROS production by which DNA/RNA can be oxidized or lipid peroxidation can progress. The redox process mediated by the potential reduction of the metal centre Cu(II)/Cu(I) might lead to the formation of oxidative species.<sup>55</sup> Intracellular ROS levels in A549 cells and excess ROS formation

by **1** were compared using the H<sub>2</sub>DCFDA staining technique. Here, it is observed that the cells treated with **1** and H<sub>2</sub>DCFDA showed more intense fluorescence than that of the control as in previous reports (Fig. 8).<sup>56</sup> This observation is an outcome of the availability of excess ROS in the complex treated cells. For instance, the cyclometalated Cu(I) pincer complexes with phosphadamantane derivatives also induced excess ROS generation that led to apoptosis in A549 cells.<sup>28</sup>

ROS-induced damage is more prominent in mitochondrial DNA than nuclear DNA.<sup>57</sup> The JC-1 assay is a very effective way to find depolarization of the mitochondrial membrane. Once the JC-1 dye enters the mitochondria of a healthy cell, it emits red fluorescence (~590 nm) of J-aggregates in healthy cells and green fluorescence (~525 nm) of their monomeric form in



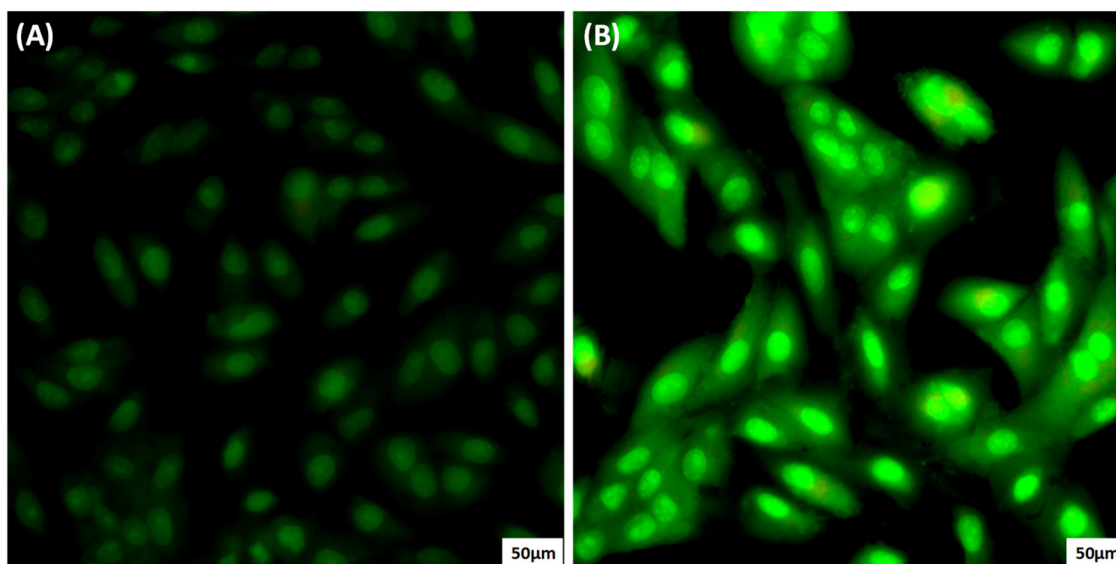


Fig. 8 H<sub>2</sub>DCFDA stained A549 cells: (A) control and (B) after being treated with **1** at the IC<sub>50</sub> concentration.

unhealthy cells. Fig. 9 explains the change in the MMP. Moreover, this change is due to the increased production of ROS which causes membrane permeabilization and results in the release of cytochrome c.<sup>58</sup> This change in the MMP is an indication of the initial phase of apoptosis.<sup>59</sup> Moreover, it is observed that the recently reported zinc(II) complex of **L3(H)** promotes the production of excess ROS and changes in the MMP.<sup>6</sup> However, the higher activity of the present copper complexes **1–3** than their zinc analogues might be due to the inherent redox-active nature of copper.

As alluded to above, the ROS formation and the change in the MMP were confirmed experimentally, and thus we propose the mechanism of anticancer activity of **1–4** with the generation of ROS in mitochondria by perturbing the function of

complex I as reported earlier.<sup>60–62</sup> Being the first enzyme of the respiratory chain, complex I has a vital role in cellular energy production.<sup>63</sup> This is possible only if **1–4** are capable of anchoring near complex I. To support this postulate, we examined molecular docking with complex I of PDB ID: 5XTD with our copper complexes.<sup>64</sup>

Interestingly, we found that **1** binds very near to the flavin mononucleotide (FMN) site of complex I (Fig. 10A); conversely, **2–4** bind away from this site with docking scores of  $-7.3$ ,  $-6.6$ ,  $-8.2$ , and  $-8.4$  kcal mol<sup>-1</sup>, respectively (Fig. S12–S14†) under the same grid box sizes.<sup>65</sup> Complex **1** binds to Gly350 (2.32 Å) and Leu349 (2.86 Å) amino acids through a strong hydrogen bonding. In complex **2**, the donor–donor bond between His376 and the amide nitrogen is very strong with a distance

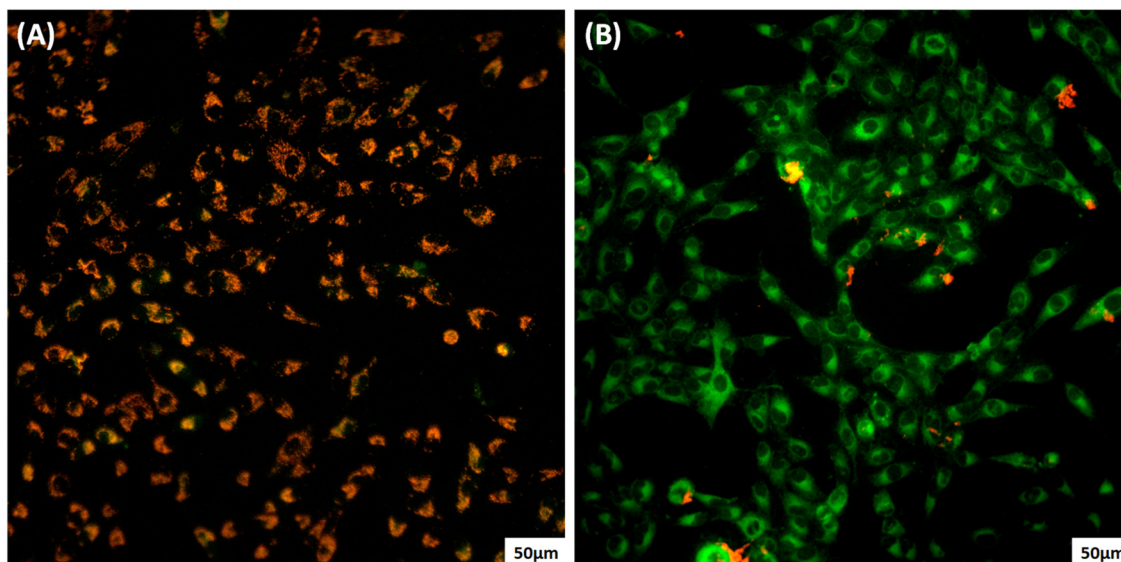


Fig. 9 JC-1 staining assay of A549 cells: (A) control and (B) after being treated with **1** at the IC<sub>50</sub> concentration.



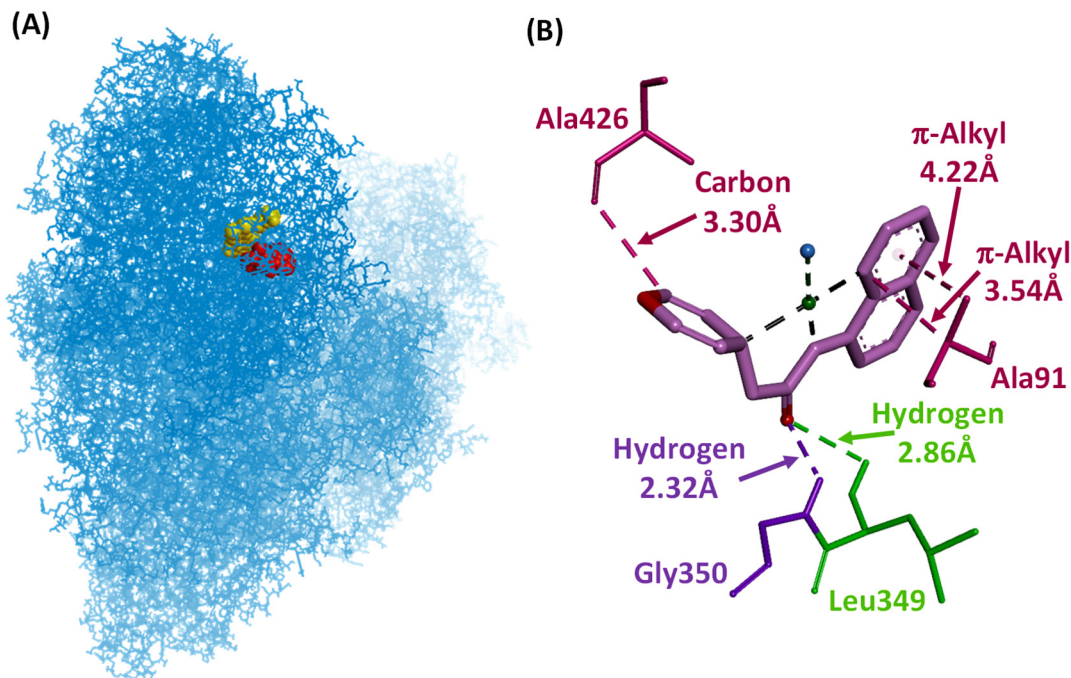


Fig. 10 (A) Molecular docked conformation and (B) interactions of **1** at the active site of 5XTD.

of 2.90 Å. Complex **3** interacted with His74 and Ala76 much more strongly with bond distances of 3.58 Å (carbon) and 3.86 Å (alkyl), respectively. A  $\pi$ - $\pi$  stacking interaction (3.80 Å) was observed with **4** and His736. In a similar way, the interaction of **4** with Val174 was observed with two strong  $\pi$ -sigma interactions with distances of 3.88 and 3.95 Å. This indicates that **1**–**4** can interact with complex I of mitochondria and possibly inhibit the activity. Specifically, the strong binding of **1** near the FMN site compared to the other complexes **2**–**4** might be the reason for its enhanced anticancer activity.

Overall, complexes **1**–**4** exhibit promising activity against A549 and MCF-7 cell lines. Mechanistic investigation reveals that the cell death of A549 is induced by the most active complex **1** because of the excess production of ROS in the mitochondria and thereby apoptosis. The observation is well supported by experimental and *in silico* studies.

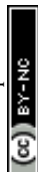
### 3.6. Lipophilicity analysis

Complex **4** and the corresponding ligand **L4(H)** were found to have a partition coefficient,  $P$  (or  $\log K_{ow}$ ), with values of 3.14 and 10.04, respectively, determined by the shake flask method.<sup>47</sup> Compounds **4** and **L4(H)** were found to be highly lipophilic compared to the other complexes **1**,  $-0.51$ ; **2**,  $1.07$ ; and **3**,  $1.83$  and ligands **L1(H)**,  $2.71$ ; **L2(H)**,  $0.67$ ; and **L3(H)**,  $0.55$ .<sup>6,7</sup> It should be noted that **1** has a slightly hydrophilic nature and shows the best activity. At the same time, **4** has higher lipophilicity which might not be good for a drug (Fig. S15†). This indicates how the lipo-hydro balance matters for drug molecules. In the present compounds, it is seen that the comparatively highly lipophilic ligands are less active than the copper complexes. The lipophilicity-anticancer activity cor-

relation of the present complexes is useful in the future to design better metal complexes in this field. Moreover, the formation constant ( $K_f$ ) of complexes **1**–**4** was calculated in methanol (Fig. S16†). The  $K_f$  value of **4** ( $K_f = (5.14 \pm 0.97) \times 10^5 \text{ M}^{-1}$ ) compared to those of the other complexes **1**–**3** is in the order  $4 < 1$  [ $(6.21 \pm 4.68) \times 10^5$ ]  $< 2$  [ $(7.19 \pm 2.42) \times 10^7$ ]  $< 3$  [ $(5.50 \pm 3.86) \times 10^9$ ].<sup>7</sup> The order of formation constant in methanol is concordant with the lipophilicity values, where the most hydrophobic **4** has the lowest  $K_f$  value.

## 4. Conclusion

Currently, growing interest in biological applications of pincer-type metal complexes proves that they can act as drugs with enhanced anticancer activity. To attain such activity, a series of 8-aminoquinoline based pincer ligands and their copper(II) complexes **1**–**4** were synthesized, characterized and studied for their anticancer activity. The complexes were very efficient against A549 lung and MCF-7 breast carcinoma cells. Interestingly, **1** was very selective against lung cancer cells with an  $\text{IC}_{50}$  value of 8  $\mu\text{M}$  and it is noteworthy that it showed no toxicity against the normal fibroblast cell line L929 ( $\text{IC}_{50} > 1000 \mu\text{M}$ ). Furthermore, the involvement of ROS in the mechanism of anticancer activity of **1** was determined by carboxy- $\text{H}_2\text{DCFDA}$  staining. Moreover, the mechanism of cell death was found to be apoptosis using the AO-EB staining assay, and through flow cytometry, it is calculated to be 25.91%. The depolarization of the MMP observed by JC-1 marking indicated elevated ROS production in the mitochondria. Furthermore, the most probable mechanism was found to be the pertur-



bation in the function of complex I of mitochondria by *in silico* studies. Overall, the studies give promising results to extend complex **1** to clinical applications.

## Author contributions

Athulya Das: data curation, formal analysis, investigation, resources, software, visualization, writing – original draft, and writing – review & editing. Muniyandi Sankaralingam: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, and writing – review & editing.

## Data availability

The data supporting this article have been included as part of the ESI.†

Crystallographic data for **1** have been deposited at the repository namely CCDC under 2311348 and can be obtained from [https://www.ccdc.cam.ac.uk/data\\_request/cif](https://www.ccdc.cam.ac.uk/data_request/cif) or by emailing [data\\_request@ccdc.cam.ac.uk](mailto:data_request@ccdc.cam.ac.uk).

## Conflicts of interest

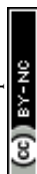
There are no conflicts to declare.

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