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Synthesis, characterization and anti-tumor activity of moxifloxacin–Copper complexes against breast cancer cell lines

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ABSTRACT

Novel moxifloxacin–copper complexes were synthesized, characterized and screened for anti-proliferative and apoptosis-inducing activity against multiple human breast cancer cell lines (hormone-dependent MCF-7 and T47D as well as hormone-independent MDA-MB-231 and BT-20). The results indicated that the parent compound moxifloxacin (**1**) does not exert any inhibitory activity against breast cancer cell lines examined. On the other hand, the copper conjugate **2** and its nitrogen adducts **3**–**5** exerted growth inhibitory and apoptosis-inducing activity against breast cancer cell lines without any substantial effect on non-tumorigenic breast epithelial cells MCF-10A at equimolar concentration, suggesting a cancer cell-specific activity. BT-20 cells were more sensitive to compounds **2** and **3**, while compounds **4** and **5** exerted significant anti-proliferative and apoptosis-inducing effects on T47D, MDA-MB-231 and BT-20 cell lines. Our results suggest that these novel compounds could be useful for the treatment of breast cancer in the future.

The fluoroquinolones are the growing group of synthetic antibiotics that exert their broad-spectrum antibacterial activities against gram-negative and gram-positive bacterial pathogens by binding to topoisomerase enzymes inducing permanent double-stranded DNA breaks, resulting ultimately in cell death.[1] Some members of this family of compounds have been shown to exert anti-tumor activity in cancer cell lines[2–5] as well as in animal model.[6] So far, the antitumor activity has been shown mainly against colon cancer,[3] bladder cancer[7] and leukemia cell lines[5] and has been linked to the topoisomerases II inhibitory activity.[5,8,9] Aranha et al. were probably the first to show that ciprofloxacin has a significant cell growth-inhibitory effect on bladder tumor cells.[10] The authors also showed that the disruption of calcium homeostasis, mitochondrial swelling and redistribution of Bax to the mitochondrial membrane were the key events in the initiation of apoptotic processes in ciprofloxacin treated bladder cancer cells.[11] Azema et al.[12] carried out screening of C-7 modified ciprofloxacin derivatives against prostate (PC-3), glioblastoma (U373-MG), colorectal (LoNo), NSCLC (A549) and breast (MCF-7) human cancer cell lines which displayed higher antitumor activity than the parent ciprofloxacin although it was not dependent upon the lipophilicity of the substituent. Additional target for the antitumor action of quinolones was thought to be the telomerase enzyme, which is activated in a vast majority of tumor cells.[13]

Metal complexation has been suggested to play an important role in the biological activities of quinolone compounds[14] where Mg^{2+} has been shown to act as a bridge between quinolone and the phosphate groups of DNA.[15] A large number of studies have been described in the literature between various quinolone derivatives and metal ions,[16] however, a thorough survey of literature on antitumor activities of metal-fluoroquinolates has revealed only a limited number of studies. Li et al.[17] have examined the anti-proliferative activity of the ternary copper complexes of ofloxacin and levofloxacin with 1,10-phenanthroline as ancillary ligand against the leukemia HL-60 as well as liver cancer HepG2 cell lines where the levofloxacin–copper complex was found to be more potent than the corresponding ofloxacin compound. Similarly, the mixed-ligand copper complexes of norfloxacin and bipyridyl/1,10-phenanthroline as ancillary ligands were found to be more active than the free ligand against HL-60 (human acute myeloid leukemia) and K562 (human chronic myeloid leukemia) cell lines, respectively.[18,19] We have also described a neutral dimeric copper complex of sparfloxacin and its phenanthroline derivative which showed considerable enhancement in its anti-proliferative activity against hormone independent BT20 breast cancer cells[20] which is

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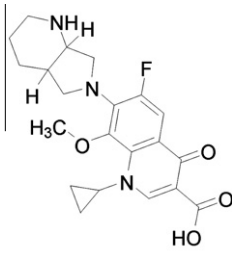


Figure 1. Structure of moxifloxacin (MOX).

interesting since high expression of topoisomerase-II has been shown to be correlated with the hormone-independent pathway.[21] Additionally, complexation with copper has been reported to inhibit the efflux mechanism effectively, thereby leading to enhanced intracellular accumulation of the quinolone drugs as reported by Jakics et al.[22]

Moxifloxacin (**1**, MOX) is 8-methoxyquinolone derivative of fluoroquinolones (Fig. 1) and belongs to the third generation of quinolone compounds, same as sparfloxacin. When administered, the compound is extensively distributed through out the body and achieves a peak serum concentration of 3.2–4.5 μg/ml with an oral dose of 400 mg of the drug. The mean half-life of the compound is 12 h while 90% of it is bioavailable.[23] The compound has been shown to inhibit DNA topoisomerase IIa (TOPO-II) which is a marker of cell proliferation in normal as well as cancerous tissues including those of breast cancer, testicular teratoma and transitional carcinoma. We were, therefore, motivated to examine the anti-proliferative activity of moxifloxacin and its copper complex against breast cancer cell lines. Since the ternary complexes of metal-quinolones were found to be more potent in our earlier work, we have also included such compounds in the present investigations. Our results indicate that ternary copper complexes of moxifloxacin are indeed very potent molecules especially against estrogen-independent (MDA-MB-231 and BT-20) breast cancer cells.

The compounds were synthesized as shown in Scheme 1.[24] The analytical data on the nitrogen adducts of copper–moxifloxacin indicated $[Cu(MOX)(L)_nCl](BF_4)\cdot xH_2O$ ($n = 1–2$, $x = 0, 4$) as the general formula for the synthesized complexes, where MOX = moxifloxacin and L = nitrogen donor ancillary ligands (Fig. 2). The molar conductivity data of all copper complexes in DMSO solvent demonstrated 1:1 electrolytic property (40–50 Ω^{-1} cm^2 mol^{-1}) indicating the presence of tetrafluoroborate counter anions.[25]

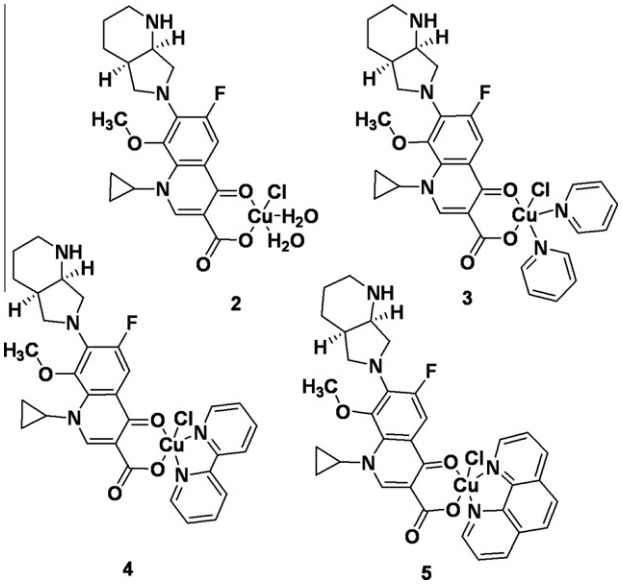
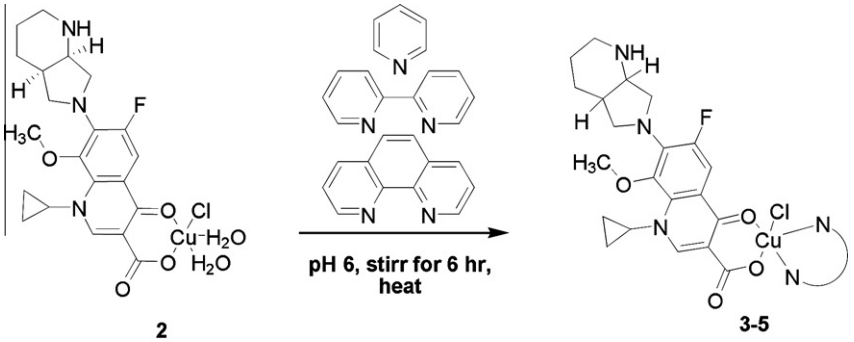


Figure 2. The proposed structures of moxifloxacin–copper complexes (**2**-**5**).

The infra red spectra of the copper complexes of moxifloxacin ligand (**1**) exhibited major changes as compared to the free ligand. The strong bands at 1708 cm^{-1} and 1622 cm^{-1} in the spectrum of moxifloxacin are assigned pyridone carbonyl and carboxyl stretches. The former is observed in other fluoroquinolones at 1716 (pefloxacin), 1718 (Gatifloxacin) and 1728 cm^{-1} (Levofloxacin), respectively.[26,27] The carboxyl is observed as a splitted absorption corresponding to asymmetric (1622 cm^{-1}) and symmetric (1375 cm^{-1}) stretches in the present compounds. In the nitrogen adducts (**3–5**) these bands are shifted to 1581–1563 and 1377–1364 cm^{-1} region. Since the frequency separation ($\Delta\nu$ = νCOOasym–νCOOsym) in the present compounds is in the range 199–204 cm^{-1}, respectively, indicative of the unidentate behavior of the carboxylate group.[28] Compound **3** exhibits characteristic strong band for the pyridyl moiety ascribed to out-of-plane bending of the ring hydrogens at 725 cm^{-1} while similar modes for the bipyridyl moiety in compound **4** ascribed are seen at 775 cm^{-1}.[29] The phenanthroline adduct **5** exhibits this band around at 727 cm^{-1}.[30] A broad band shown by all compounds at 3350–3420 cm^{-1} is indicative of the presence of the lattice-held water molecules, whereas the strong absorption band around 1031–1058 cm^{-1} confirms the presence of the BF_4^{-1} anion.[31]



2 - Copper complex of 1
3 - Copper complex of 1 with pyridyl as ancillary ligand
4 - Copper complex of 1 with bipyridyl as ancillary ligand
5 - Copper complex of 1 with phenanthroline as ancillary ligand

Scheme 1. Schematic representation of synthesis of moxifloxacin–copper complexes (**2–5**).

Table 1
X-band ESR parameters on copper complexes of moxifloxcin

Compounds	$g_{\|\|}$	$g_{\perp}$	$A_{\|\|}$		f (cm)
			mT	$\times 10^{-4}$ cm^{-1}	
2	2.32	2.04	17.81	192	120
3	2.30	2.04	18.88	203	113
4	2.35	2.03	17.95	197	116
5	2.35	2.04	17.29	190	124

Table 2
Electrochemical data for complexes (**2**–**5**)

Compounds	E_{pc} (v)	E_{pa} (v)	$E_{1/2}$ ($Cu^{2+/1+}$)
[Cu(moxi)(H_2O)$_2$Cl]BF_4 (**2**)	+0.23	+0.44	+0.34
	−0.61	−0.06	
[Cu(moxi)(py)$_2$ Cl]BF_4·H_2O (**3**)	+0.23	+0.50	+0.32
	−0.70	−0.29	
[Cu(moxi)(bipy)Cl]BF_4·4H_2O (**4**)	−0.09		+0.11
	−0.71	+0.31	
	−1.65	−0.05	
[Cu(moxi)(phen)Cl]BF_4·4H_2O (**5**)	+0.05		+0.14
	−0.63	+0.23	
	−1.83	−0.25	

E_{pc} = cathodic potential and E_{pa} = anodic potential.

The electronic spectra of the complexes (**2**–**5**) in dimethyl sulphoxide (DMSO) solvent reveal the intra-ligand absorptions in the UV region and ligand-to-metal charge transfer transitions around 360 nm. The compounds exhibit a broad absorption in the range 650–900 nm attributed to d–d transition for Cu (II) atom in a distorted square pyramidal environment.[32,33] The magnetic moments of all complexes are found to fall in the range 1.75–1.87 B.M. which is close to the spin-only values expected for $S = 1/2$ system.[34] Absence of EPR signal corresponding to the $M_s = +2$ transition at the half-field region even at high gain suggests monomeric nature of the present complexes.[35] All nitrogen ad-

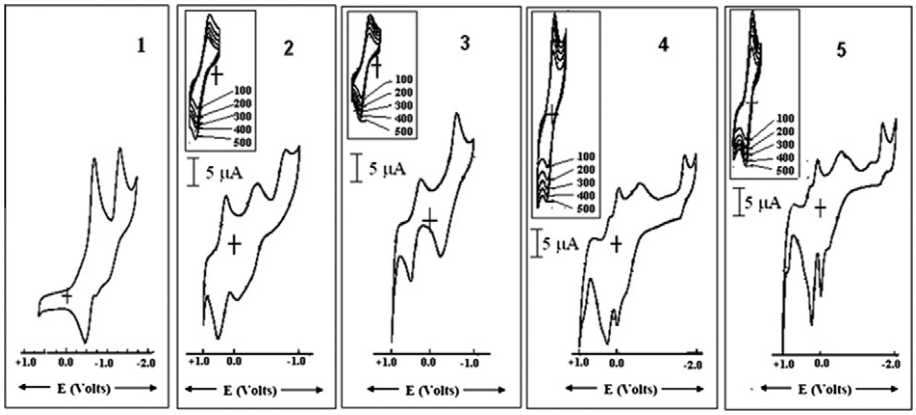


Figure 3. Cyclic voltammograms of a 10^{-3} M solutions of compounds (**2**–**5**) in DMSO solutions at a scan range of (100 mV s^{-1}). **1**: Moxifloxacin.

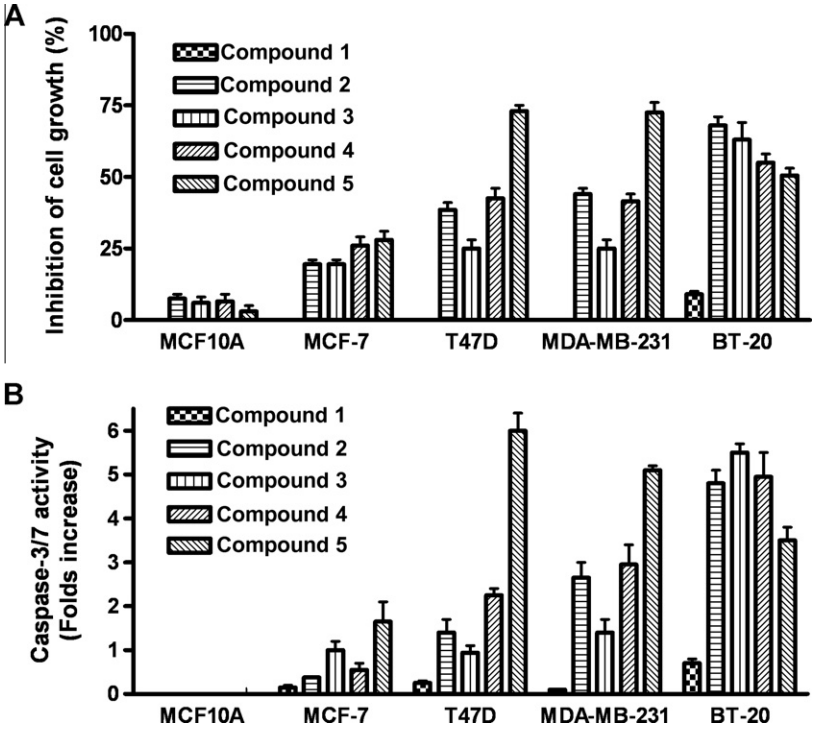


Figure 4. (A) Anti-proliferative and (B) apoptosis-inducing activity of compounds (**1**–**5**) (5 μM) against non-tumorogenic MCF-10A and breast cancer cell lines, MCF-7, T47D, MDA-MB-231 and BT-20. All values are expressed relative to activity of vehicle control (DMSO). Values are expressed as mean ± SE and are representative of three independent repeats.

Table 3
IC_{50} values (μM) of compounds (**1**–**5**) against breast cancer cell lines, as determined by MTT assay[41]

Cell line	Compound				
	1	**2**	**3**	**4**	**5**
MCF-10A	—	—	—	—	—
MCF-7	—	24.2 ± 2.3	18.4 ± 1.9	16.7 ± 2.2	18.1 ± 2.6
T47D	—	7.6 ± 0.3	19.5 ± 1.2	7.4 ± 0.2	1.6 ± 0.1
MDA-MB-231	—	7.1 ± 0.2	20 ± 1.1	5.8 ± 0.4	1.7 ± 0.3
BT-20	—	2.0 ± 0.1	3.4 ± 0.2	4.0 ± 0.3	4.9 ± 0.2

All values are mean values ± SE from three independent experiments. Dose-dependent effect of compounds was tested against breast cancer cell lines by evaluating doses ranging from 1 to 50 μM. 50% killing of MCF-10A normal breast epithelial cells was not achieved by any of the compounds at any of the doses tested. Within the range of tested concentrations, IC_{50} values of compound **1** were not observed against any of the tested cells.

ducts exhibit axial EPR spectra of square pyramidal species with g_{II} varying from 2.30 to 2.35 and A_{II} ranging from 190 to $203 \times 10^4\,cm^1$ From the observed $g_{\perp}$ values for the present compounds (2.04) it is clear that $g_{||} > g_{\perp}$ which suggests that the unpaired electron is predominantly in the d_{x2y2} orbital giving $^2B_{1g}$ as the ground state (Table 1).[36]

The cyclic voltammographic profiles of all synthesized copper complexes in DMSO were studied in the range +1.0 to −1.5 V which exhibit two peaks at −0.75 and −1.30 V in the cathodic region (Fig. 3) which are ascribed to the reduction of diazbicylononyl and pyridone moieties, respectively.[37,38] The metal-based peak corresponding to a reversible $Cu^{2+/1+}$ redox couple is observed for all complexes in the range of +0.11 to +0.34 V (Table 2). The range for the corresponding ciprofloxacin complexes was found to be −0.06 to +0.11V[39] which suggests that the more facile copper redox-couple may contribute more to the biological activities of these compounds.

The anti-proliferative activities of **1** and its copper complexes were evaluated against four breast cancer cell lines[40,41] representing different receptor status viz. MCF-7, T47D, MDA-MB-231 and BT-20, along with the normal breast epithelial MCF-10A cell line, respectively (Fig. 4A). The parent ligand as well as its copper complexes did not significantly inhibit the proliferation of non-tumorogenic MCF-10A breast epithelial cells but had varying effects on cancer cell lines suggesting a cancer cell-specific action. Various concentrations of drugs were used to calculate reported IC_{50} values and a representative dose (5 μM) is shown in Figure 4A. Interestingly, moxifloxacin itself did not exhibit anti-proliferative effect against any of the breast cancer cell lines examined. However, when complexed with copper it showed differential anti-proliferative activity against various breast cancer cell lines, as evident by the range of IC50 values (Table 3). Among the nitrogen-adducts, the phenanthroline adduct **5** consistently showed higher anti-proliferactive effects against all breast cancer cell lines with particularly high anti-proliferative activity against T47D and MDA-MB-231 cells (Fig. 4A). Since induction of apoptosis represents a common mechanism by which anticancer agents exert their biological effects, we tested the apoptosis-induction activity of our test compounds.[42] Increased activity of caspases is a hallmark of apoptosis. Accordingly, we tested the ability of compounds **1-5** to induce the activity of caspases-3/7 and the representative results (at 5 μM dose) are shown in Figure 4B. Again, compounds **2**–**5** were found to induce apoptosis in cancer cell lines to different extent, with no effect on MCF-10A cells. Similar to anti-proliferative studies, BT-20 cells were observed to be particularly sensitive to treatment with almost all the compounds and compound **5** was found to induce significant apoptosis in T47D and MDA-MB-231 cells.

The present work clearly demonstrates that nitrogen adducts of the moxifloxacin–copper complex are endowed with differential anti-proliferative activity towards breast cancer cell lines without any toxicity towards non-tumorogenic breast epithelial cells. These preliminary results are exciting and further elucidation of the biological activity of these novel compounds against breast cancer as well as other cancers is warranted.

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24. All chemical substances were of analytical reagent (AR) grade and were used without further purification. Moxifloxacin hydrochloride, the product of Torrent pharmaceutical Ltd (India), was purchased from local pharmacy whereas 1,10-phenanthroline (E.Merck, India Ltd), 2,2′-bipyridine (S.D. Fine-Chem Ltd), pyridine (Thomas Baker Chemicals Ltd, Bharat Mahal, Marine Drive, Mumbai) and $[Cu(MeCN)_4]BF_4$ were prepared according to standard procedure. Magnetic susceptibilities of the moxifloxacin–copper complexes were measured at 300 K on a Faraday balance having field strength of 7000 KG by using Hg [Co (SCN) $_4$] as a calibrant. Electronic spectra were recorded on Genesys–UV–vis–NIR spectrophotometer in the 270–1100 nm range in DMSO solvent. Cyclic voltammetric measurement were done in DMSO on a Bioanalytical system BAS CV—27 instrument with an XY—recorder using a Pt disc as working electrode against SCE and Pt wire as an auxiliary electrode. Et_4NClO_4 (TEAP) was used as the supporting electrolyte. Elemental analysis was carried out using a CHNS/O Analyzer Perkin–Elmer PE 2400 series II. IR spectra were recorded as Nujol mulls in the 4400–450 cm^{-1} range on Perkin–Elmer 1615 FT-IR spectrophotometer.
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40. *Cell culture*: Breast cancer cell line T47D was maintained in RPMI culture medium (Invitrogen) while the breast cancer cell lines MCF-7, MDA-MB-231 and BT-20 were maintained in DMEM medium (Invitrogen). Both the culture media contained penicillin (50 U/ml), streptomycin (50 μg/ml) and 10% fetal calf serum. The non-tumorigenic breast epithelial cell line, MCF-10A (considered to be normal breast epithelial cells), was propagated in DMEM/F12 (Invitrogen, Carlsbad, CA) supplemented with 5% horse serum, 20 ng/ml EGF, 0.5 μg/ml hydrocortisone, 0.1 μg/ml cholera toxin, 10 μg/ml insulin, 100 units/ml penicillin, and 100 μg/ml streptomycin. All cells were cultured in a 5% CO_2-humidified atmosphere at 37 °C.
41. *Cell growth inhibition studies by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay*: Cells (3×10^3/well) were seeded in 96-well culture plates. Each treatment had eight replicate wells and, moreover, each experiment was repeated at least three times. Test compounds were dissolved in DMSO and added to cells 24 h after seeding. At the end of treatment, MTT (0.5 mg/ml) was added and plates incubated at 37C for 2 h followed by replacement of media with DMSO at room temperature for 30 min. Ultra Multifunctional Microplate Reader (TECAN) was used to record the absorbance.
42. *Homogeneous caspase-3/7 assay for apoptosis*: Caspase-3/7 homogeneous assay was performed using a kit from Promega (Madison, WI). Cells were treated with indicated compounds or DMSO control. After treatment, 100 μl Apo-ONE® caspase-3/7 reagent was added and plates were shaken for 2 min, followed by incubation at room temperature for 3 h. The fluorescence was then evaluated using ULTRA Multifunctional Microplate Reader (TECAN) at excitation/emission wavelengths of 485/530 nm. Activation of caspase-3/7 by DMSO alone was also tested and the activation by test compounds is expressed as folds change compared to activation by vehicle (DMSO) control.