Synthesis, X-ray Structure, Antimicrobial and Anticancer Activity of a Novel [Ag(ethyl-3-quinolate)$_2$(citrate)] Complex

Mezna Saleh Altowyan [1], Mostafa A. El-Naggar [2,*], Morsy A. M. Abu-Youssef [2], Saied M. Soliman [2,*], Matti Haukka [3], Assem Barakat [4] and Ahmed M. A. Badr [2,*]

[1] Department of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; msaltowyan@pnu.edu.sa

[2] Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt; morsy5@alexu.edu.eg

[3] Department of Chemistry, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland; matti.o.haukka@jyu.fi

[4] Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia; ambarakat@ksu.edu.sa

* Correspondence: mostafa.elnaggar@alexu.edu.eg (M.A.E.-N.); saied1soliman@yahoo.com (S.M.S.); ahmed_badr@alexu.edu.eg (A.M.A.B.); Tel.: +201012463029 (M.A.E.); +201005002949 (A.M.A.B.)

Crystal structure determination details

The crystal of **[Ag(Et3qu)2(citrate)]** was immersed in cryo-oil, mounted in a loop, and measured at a temperature of 120 K. The X-ray diffraction data was collected on a Rigaku Oxford Diffraction Supernova diffractometer using Cu Kα radiation. The *CrysAlisPro* software package was used for cell refinement and data reduction. An analytical absorption correction (*CrysAlisPro*) was applied to the intensities before structure solution. Structure was solved by intrinsic phasing (*SHELXT*) method. Structural refinement was carried out using *SHELXL* software with *SHELXLE* graphical user interface. The two -O-CH_2-CH_3 groups and one oxygen atoms (O8) were disordered over two sites. Oxygens O3 and O3B were constraint to share the same location with equal anisotropic displacement parameter. Oxygen O4B was restrained with so that its U_{ij} components approximate to isotropic behavior. The OH hydrogen atoms were located from the difference Fourier map and refined isotropically. Other hydrogen atoms were positioned geometrically and constrained to ride on their parent atoms, with C-H = 0.95-0.99 Å and U_{iso} = 1.2-1.5 U_{eq}(parent atom).

Method S1

Antimicrobial activity Assay

The microbial strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antimicrobial profile was tested against Gram-positive bacterial species (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacterial species (*Escherichia coli, Proteus vulgaris*), as well as against fungi including one filamentous fungus (*Aspergillus fumigatus*) and one yeast species (*Candida albicans*) using a modified well diffusion method. Briefly, 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria or 10^5 cells/mL for fungi. One hundred µL of microbial suspension was spreaded onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method. One hundred µL of each sample (at 10 mg/mL) was added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 h at 37 °C (for bacteria and yeast) and for 48 h at 28 °C (for filamentous fungi). After incubation, the microorganism's growth was observed. The resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. Solvent

controls (DMSO) were included in every experiment as negative controls. DMSO was used for dissolving the tested compound and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms. Positive controls were also performed using Gentamycin as standard antibacterial drugs and Ketoconazole as standard antifungal drug. All the biologically active samples were subjected to determinate the MIC by the broth mirodilution method. After incubation, the lowest concentration showing complete inhibition of growth was recorded as the MIC of the respective sample [S1].

Method S2

Evaluation of Cytotoxic Effects against A-549 cell line

Mammalian cell line A-549 (human Lung Carcinoma) cells was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Dimethyl sulfoxide (DMSO), MTT and trypan blue dye was purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

Cell line Propagation:

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/mL Gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

Cytotoxicity evaluation using viability assay:

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red then 10 µL of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µL aliquot of the media was removed from the wells, and 50 µL of DMSO was added to each well and mixed thoroughly with the pipette and incubated at

37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) [S3].

References

[S1] CLSI; Clinical and Laboratory Standards Institute, 2012, Twentieth informational supplement. M100-S22. Wayne: PA.

[S2] Mosmann, T. (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods; 65: 55-63.

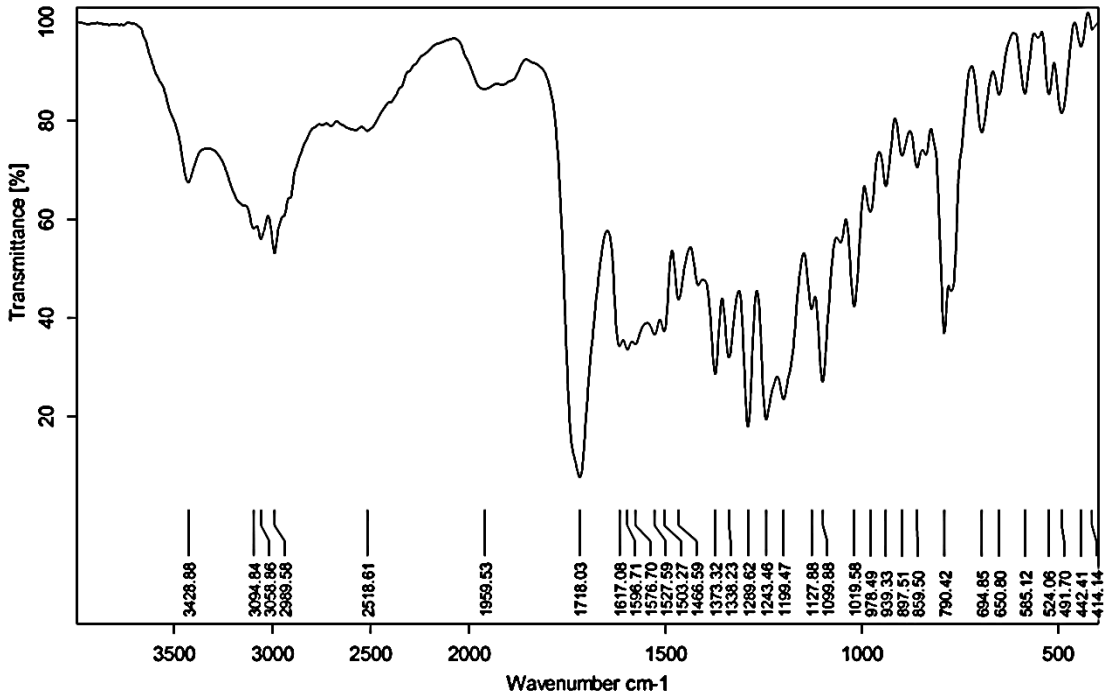

Figure S1. The FTIR spectrum of the complex, **[Ag(Et3qu)$_2$(citrate)]**.

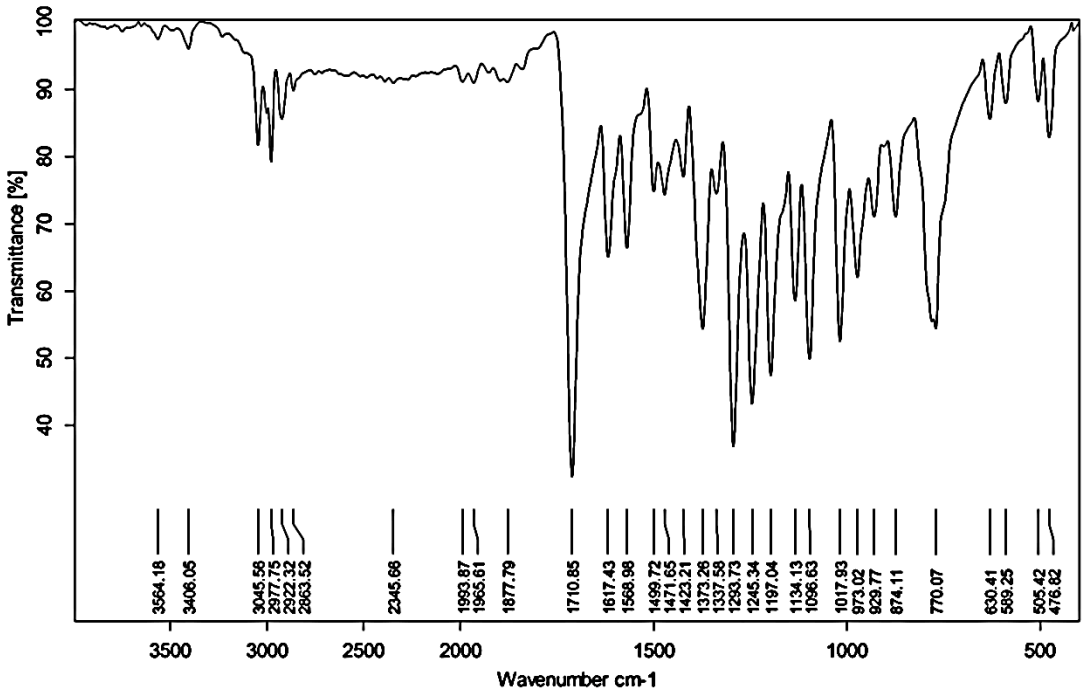

Figure S2. The FTIR spectrum of the free ligand, **Et3qu**.

Table S1. MTT assay for the Ag(I) complex against MCF-7 cell line.

Sample conc. (µg/mL)	Viability %	Inhibitory %	S.D. (±)
500	1.33	98.67	0.31
250	3.17	96.83	0.29
125	5.89	94.11	0.15
62.5	8.42	91.58	0.48
31.25	14.05	85.95	0.61
15.6	19.87	80.13	0.59
7.8	27.04	72.96	0.62
3.9	36.59	63.41	1.23
2	47.12	52.88	2.46
1	70.84	29.16	1.08
0.5	81.23	18.77	2.95
0.25	88.40	11.60	0.64
0	100	0	0

Table S2. MTT assay for **Et3qu** against MCF-7 cell line.

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	4.23	95.77	0.35
250	8.97	91.03	0.59
125	19.52	80.48	0.64
62.5	30.65	69.35	1.31
31.25	48.96	51.04	2.18
15.6	77.58	22.42	2.36
7.8	92.31	7.69	1.59
3.9	99.42	0.58	0.84
2	100	0	0
1	100	0	0
0.5	100	0	0
0.25	100	0	0
0	100	0	0

Table S3. MTT assay for the Ag(I) complex against A-549 cell line.

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	0.69	99.31	0.27
250	2.41	97.59	0.15
125	4.28	95.72	0.46
62.5	6.97	93.03	0.31
31.25	11.83	88.17	0.59
15.6	17.25	82.75	1.37
7.8	22.69	77.31	0.83
3.9	30.45	69.55	0.95
2	39.73	60.27	0.63
1	48.20	51.80	1.42
0.5	69.94	30.06	1.08
0.25	78.03	21.97	1.79
0	100	0	0

Table S4. MTT assay for **Et3qu** against A-549 cell line.

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
500	2.87	97.13	0.45
250	5.49	94.51	0.73
125	11.98	88.02	0.84
62.5	24.36	75.64	1.72
31.25	37.08	62.92	1.98
15.6	61.42	38.58	2.34
7.8	78.19	21.81	1.27
3.9	89.03	10.97	0.61
2	97.16	2.84	0.82
1	100	0	0
0.5	100	0	0
0.25	100	0	0
0	100	0	0