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Synthesis, Characterization and Cytotoxic Activity of 8-Hydroxyquinoline Derivatives

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ABSTRACT

The cytotoxic effects of synthesized quinoline derivatives were tested against the leukemia derived Nalm6 cell lines. The preliminary cytotoxicity screening program revealed that the investigated compounds induced 50% inhibition of the cell at micromolar concentrations and thus could be considered as biologically active. The compound of 5-chloro-8-hydroxyquinolinium chloride proved superior to the remaining agents. The synthesized compounds were characterized by ^{1}HNMR, ^{13}CNMR and Mass spectroscopy. Moreover, the crystal structure of 5-chloro-8-hydroxyquinolinium chloride was solved which confirms the several hydrogen bonding in the solid state of this compound that stabilized crystal packing.

Keywords: Cytotoxic activity, 8-Hydroxyquinoline derivatives, Crystal structure, Hydrogen bonding

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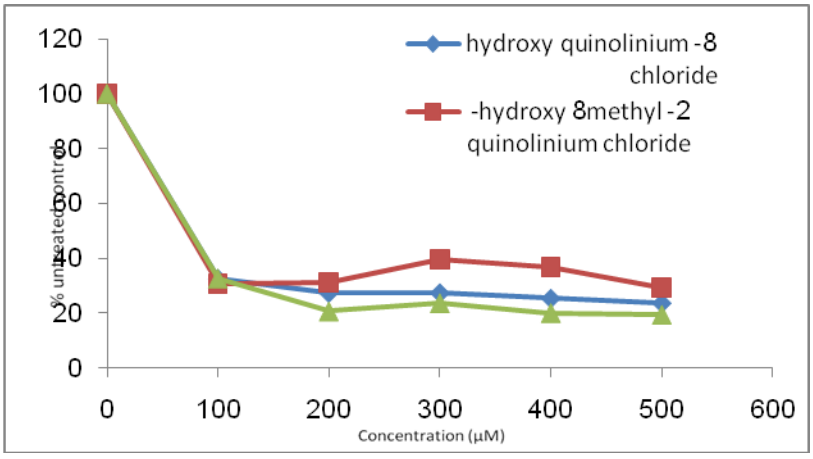
INTRODUCTION

8-Hydroxyquinoline and its derivatives are well known for their bioactivities and chelating abilities in inorganic chemistry[1-4]. The biological activities of these compounds have been correlated with their capacity to chelate reagents in analytical chemistry and radiochemistry for metal ion extraction. These compounds are interesting because they can perform as structurally related subunits in important biomolecules or biochemical process, which show strong cytotoxic and antimicrobial properties; also represent the main component in some bactericide, fungicide and antimalarial drugs. There is considerable interest in proton transfer[5-7] of both organic and inorganic chemistry of these compounds.

As already anticipated, the low water solubility of previously synthesized quinolines limits their further development as potential drug candidates. In the present study, we decided to analyze the water solubility profile of new quinoline derivative selecting those with the best compromise between antiproliferative activity and appropriate water solubility. In principle, the aqueous solubility of a chemical compound may be defined as the maximum amount of this chemical that dissolves in water at a specified temperature and pH.

Interestingly, among the synthesized compounds 5-chloro-8-hydroxyquinolinium chloride had shown the highest antiproliferative activities against our cell line panel and the best water solubility profile, as reported in Figure 1.

Figure 1. Cytotoxic effects of the tested compounds against the human leukemia Nalm6 after 72 h



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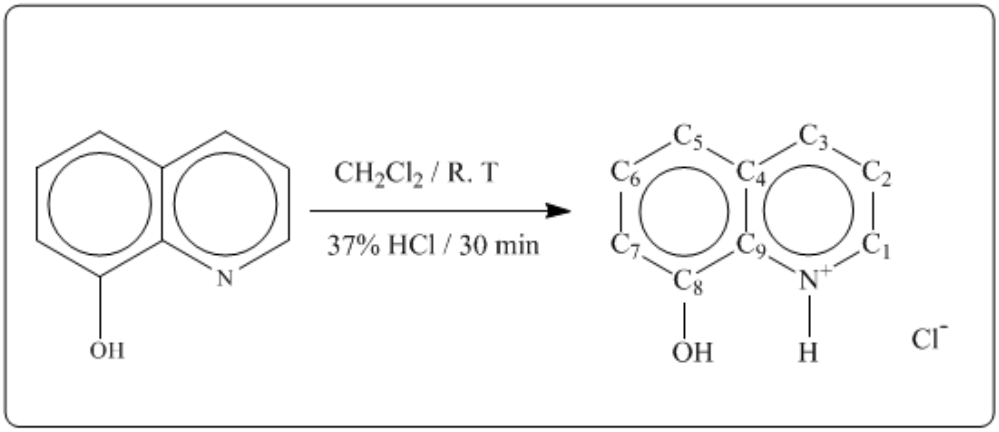
EXPERIMENTAL PROCEDURE

All solvents were obtained from commercial sources and used without further purification. 5-chloro 8-hydroxyquinoline (>99%) and hydrochloric acid (37%) were purchased from Merck chemicals. Melting points are uncorrected and were obtained with Electrothermal 9200 melting point apparatus. Infrared spectra from 250 to 4000 cm^{-1} of solid samples were taken as 1% dispersion in CsI pellets using a Shimadzu-470 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature in D_2O on a Bruker AVANCE 300 MHz. The NMR spectra are referenced to Me_4Si as external standard. Elemental analysis was performed using a Heraeus CHN–O Rapid analyzer.

2.1 Synthesis of 8-hydroxyquinolinium chloride, 2-methyl-8-hydroxyquinolinium chloride, 5-chloro-8-hydroxyquinolinium chloride

To (5.56 mmol) of a (a = 8-hydroxyquinoline, b = 2-methyl8-hydroxyquinoline, c = 5-chloro 8-hydroxyquinoline) dissolved in 20 ml dichloromethane, 6 mmol of 37% hydrochloric acid was added dropwise with cooling. The resulting suspension was stirred for 30 min. The obtained solid was filtered off and then dried over P_2O_5.

0.8 g of 8-hydroxyquinolinium chloride resulted as a yellow powder, mp=225°C ; ^{1}H NMR (500 MHz, D_2O):δ_H (ppm) 8.03-8.04 (d ,5Hz, H_1), 8.01-8.02(d , 5Hz, H_3), 7.78-7.76 (t, 5.5Hz,H_2), 7.74-7.72 (t, 5.5Hz, H_6) 7.63-7.62 (d, 5Hz,H_7), 7.62-7.61 (d, 10Hz, H_5), Scheme 1.



Scheme .1 Synthesis of 8-hydroxyquinolinium chloride with atom numbering

1 g of 2-methyl-8-hydroxyquinolinium chloride as light yellow powder ,mp=256 °C; ^{1}H NMR (500 MHz, D_2O): δ_H (ppm) 2.5 (s , H_1), 8.93-8.95 (d , 10Hz, H_3), 7.89-7.91 (t, 5 Hz,H_2), 7.68-7.70 (d, 10Hz, H_5) 7.57-7.59 (d, 10Hz ,H_7), 7.60-7.63 (t ,7.5Hz, H_6); Scheme 2.

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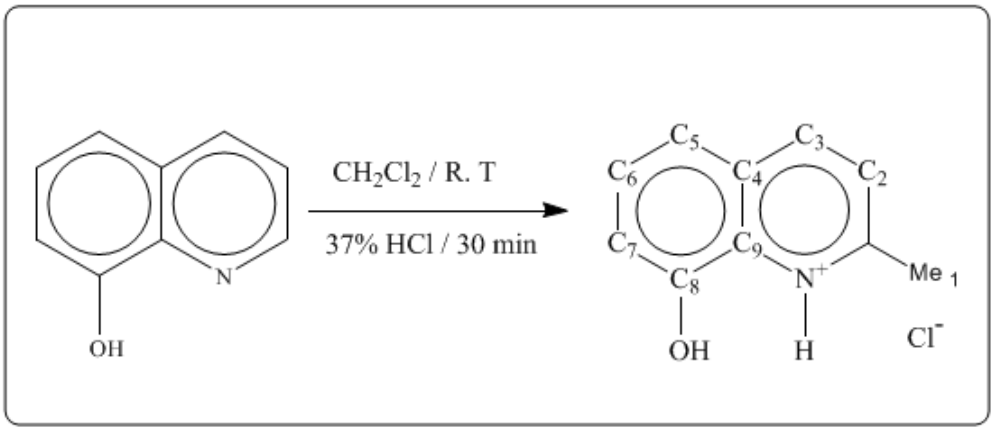

Scheme .2 Synthesis of 2-Methyl 8-hydroxyquinolinium chloride with atom numbering

1.09 g of 5-chloro-8-hydroxyquinolinium chloride resulted as a shiny yellowish-green micro-crystals, mp>304°C (dec), (84.7% yield). MS, m/z (%): 215 ; ^{1}H NMR (300 MHz, D_2O): δ_H (ppm) 9.37-9.40 (d ,9Hz, H_1), 9.17-9.19 (d ,6Hz, H_3), 8.21-8.26 (t ,7.5Hz, H_2), 7.91-7.93 (d, 6Hz,H_6), 7.44-7.47 (d, 9Hz, H_7), ^{13}C-NMR (300 MHz, D_2O): δ_C (ppm) 147.71(C_1), 144.11(C_8), 143.45 (C_9),130.59 (C_3),130.14(C_6) 127.73(C_4), 122.94(C_2), 121.09(C_5),115.85(C_7), Scheme 3.

Scheme 3.Synthesis of 5-chloro-8-hydroxyquinolinium chloride with atom numbering

RESULT AND DISCUSSION

Structure determination

The crystallographic data was collected with a Nonius Kappa CCD diffractometer, using graphite-monochromated Mo-Kα radiation of 0.71073 Å. A yellowish-green block with a dimension of 0.10 × 0.11 × 0.08 mm was mounted. Data were collected at a temperature of 293(2) K to a maximum 2θ value of 53.98°. The numerical absorption coefficients, μ, for Mo-Kα radiation is 0.683 mm^{-1}. The structures were solved by direct methods[8] and subsequent differences Fourier map and then refined on F^2 by a full-matrix least-squares procedure using

anisotropic displacement parameters[8]. Cell refinements were performed using the Denzo-SMN crystallographic software package[9]. A summary of the crystal data, experimental details and refinement results is given in Table 1.All the bond lengths and angles are in their expected range. Selected bond lengths and angles are summarized in Table 2.

Table 1. Crystal data and structure refinement for the title compound

CCDC deposit no.	786214
Molecular formula	$C_9H_7Cl_2NO$
Molecular weight	216.06
Temperature (K)	293(2)
Radiation λ	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
a/Å	7.3971(15)
b/ Å	7.6524(15)
c/ Å	8.4460(17)
V/ $Å^3$	443.83(18)
Z	2
D_{calc}(g cm^{-3})	1.617
Crystal size (mm)	0.10×0.11×0.08
Crystalcolour	Yellowish-green
Absorption coefficient (mm^{-1})	0.683
F(000)	220
Reflections collected/unique	4031/ 1932 [R_{int}=0.036]
Range/indices (h, k, l)	-9,9; -9,9; -10,10
No. of observed data, I > 2σ(I)	1482
No. of restraints	0
Goodness of fit on F^2	1.04
R_1, wR_2 [I≥2 σ(I)][a]	0.0448, 0.1134
R_1, wR_2 (all data)	0.0630, 0.1240

[a]*R* values are defined as: $R_1 = \Sigma||F_O|-|F_C|| / \Sigma|F_O|$,$wR_2 = [\Sigma[w(F_O^2-F_C^2)^2] / \Sigma[w(F_O^2)^2]]^{1/2}$

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Table 2. Selected bond lengths (Å) and angles (˚)

Cl2-C3	1.741(2)	N1-C11	1.366(3)
O1-H1	0.83(3)	O1-C6	1.351(3)
N1-C7	1.329(3)	N1-H11b	1.01(4)
C6-O1-H1	109(2)	Cl2-C3-C4	119.51(19)
C7-N1-C11	122.9(2)	Cl2-C3-C10	119.49(18)
C7-N1-H11b	115.1(19)	O1-C6-C11	115.4(2)
C11-N1-H11b	122.0(19)	O1-C6-C9	126.3(2)

Symmetry code: b= x,1+y,-1+z

Water solubility profile

The cell-killing activities of the quinoline derivative compounds were evaluated through a series of controlled cytotoxicity assays and against a number of the leukemia derived Nalm6 cell lines .

In these cytotoxicity experiments, a DMSO solution of compounds were used . It is important to note, however, that the amount of the DMSO cosolvent was so small that its overall effect on the cells might be considered negligible. Still, to ensure elimination of such effects, a blank DMSO control was included in each assay.

It is expected that the improvement in the synthesized compound system cytotoxicity was due to the water solubility. Complete solubilization might have provided the cells with a longer “effective exposure time ” to the drug.

Study of synthesized compound in solution state; 8-hydroxyquinolinium chloride , 2-methyl-8-hydroxyquinolinium chloride, 5-chloro-8-hydroxyquinolinium chloride

We have not seen the hydroxyl group and the proton bonded to nitrogen (N^+-H) in the ^{1}H-NMR spectrum of the title compounds because they are changeable with deuterium of D_2O solvent. The other protons were appeared in expected positions.

Solid state of 5-chloro-8-hydroxyquinolinium chloride

The yellowish-green block crystals of the salt were obtained by recrystallization in MeCN. The salt crystallizes in triclinic space group Pī with two molecules in the unit cell. The ORTEP view of the compound was depicted in Figure 2.

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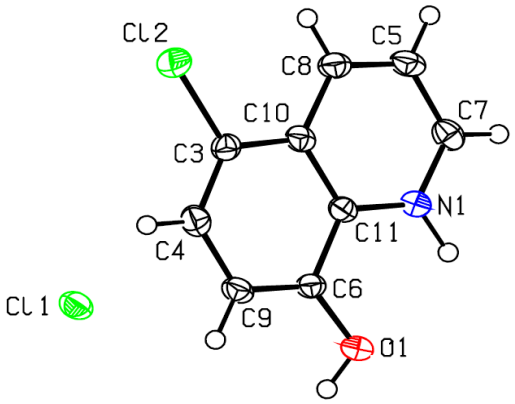

Figure 2.The labeled diagram of 5-chloro-8-hydroxyquinolinium chloride.Thermal ellipsoids are at 50% probability level.

The packing of this salt was stabilized with several interactions such as hydrogen bonding (Figure 3,4).

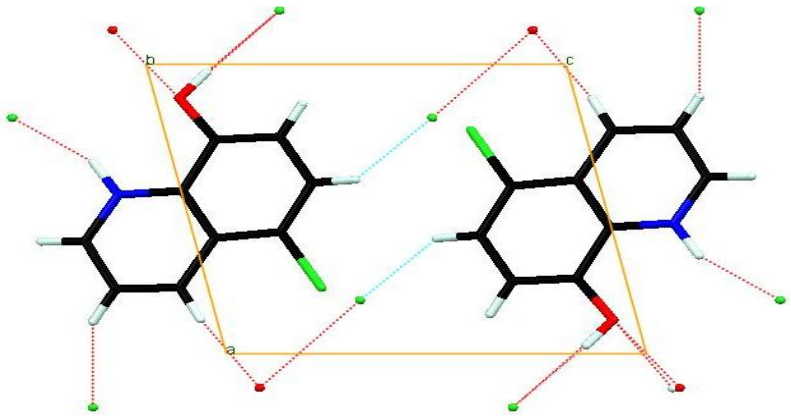

Figure 3.Packing diagram of the title compound along *b* axis. Hydrogen bonding, weak O…O and O…Cl interactions were shown by dashed lines.

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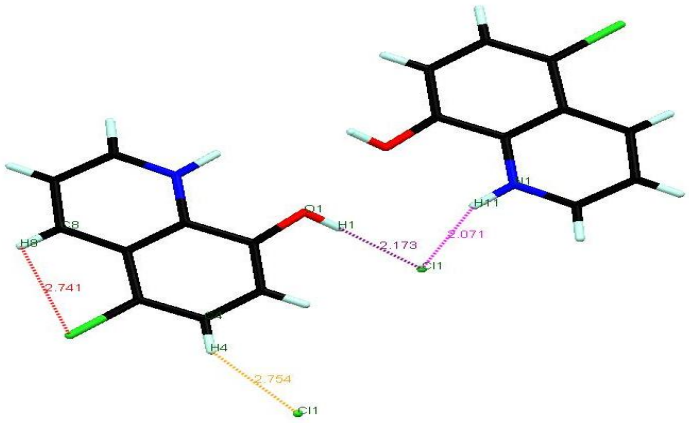

Figure 4.C-H…Cl and O-H…Cl hydrogen bondings in the packing of the salt with atom numbering and bond distances.

Weak non-covalant C-H…Cl hydrogen bondings which are summarized in Table 3, have important role in formation of packing. Also, there are π…π stacking interactions in the lattice, where the pyridine-like ring of the salt unit are stacked over the flanking phenyl-like ring of the adjacent salt molecule. The distance between the planes of the pyridine of one unit and the flanking phenyl of the next molecule is 3.40A˚ (3.61A˚ centroid–centroid) (Fig.5).

Table 3. Hydrogen bonds (Å and °) for 5-chloro-8-hydroxyquinolinium chloride

D-H…A	D-H	H…A	D…A	∠DHA	Symmetry code
O1-H1…Cl1	0.83(3)	2.17(3)	3.0015(18)	173(3)	-x,1-y,1-z
N1a- H11…Cl1	1.01(4)	2.07(4)	3.028(2)	158(3)	x,-1+y,1+z
C4-H4…Cl1	0.9300	2.7500	3.545(3)	143	
C8-H8…Cl2	0.9300	2.7400	3.113(3)	105.00	

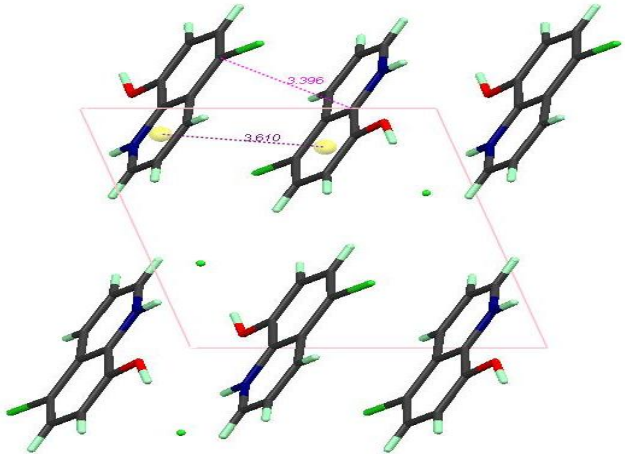

Figure 5. weak π…π interactions between 8-hydroxyquinoline moieties in the crystal packing of 5-chloro-8-hydroxyquinilium chloride along *a* axis.

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In vitro cytotoxicity

The cytotoxic effects of the three newly synthesized compounds of quinoline derivatives against the human leukemia pro B lymphocyte Nalm 6 cells were determined using the standard MTT-dye reduction assay for cell viability. The cytotoxic potential of the free ligands was evaluated as well. The retrieved MTT-formazan absorption values are summarized in Table 4. The 72 h exposure of both cell lines with the tested compounds resulted in a concentration-dependent reduction of cell viability as assessed by the MTT-dye reduction assay, which enabled the construction of concentration–response curves. In addition the corresponding IC50 values were derived in order to allow a quantitative merit for assessment of the relative potencies of the agents under investigation (Table 5).

Table 4.Spectrophotometrical data from the MTT assay concerning the cytotoxic effects of the newly synthesized compounds on Nalm6 leukemic cells

Concentration (μM)	MTT- formazan absorption (580 nm)		
	a	**b**	**c**
0	0.198 ± 0.005	0.198 ± 0.005	0.198 ± 0.005
100	0.131 ± 0.022	0.123 ± 0.002	0.132 ± 0.035
200	0.11 ± 0.011	0.125 ± 0.025	0.084 ± 0.014
300	0.11 ± 0.024	0.159 ± 0.007	0.096 ± 0.004
400	0.102 ± 0.04	0.147 ± 0.178	0.081 ± 0.002
500	0.095 ± 0.026	0.117 ± 0.055	0.079 ± 0.003

a: 8-hydroxy quinolinium chloride,b: 2-methyl 8-hydroxy quinolinium chloride, c: 5-chloro 8-hydroxy quinolinium chloride

Table 5.Relative potency of the compounds after 72 h exposure (MTT-dye reduction assay)

Compound	IC_{50} value (μM)
Nalm 6	
a	74.16
b	72.17
c	74.4

a: 8-hydroxy quinolinium chloride,b: 2-methyl 8-hydroxy quinolinium chloride, c: 5-chloro 8-hydroxy quinolinium chloride

Cell culture maintenance, drug solutions and treatment

The cytotoxic activity of the tested zirconium complexes was evaluated against the human pro B lymphocyte leukemia Nalm6 cells. The cell lines exploited here in were supplied from the German Collection of Microorganisms and Cell Cultures. They were maintained as suspension-type cultures in a controlled environment (RPMI-1640 medium, supplemented with 10% heat inactivated fetal calf serum, at 37 °C in a 'Heraeus' incubator with 5% CO_2 humidified atmosphere). In order to maintain the cells in log phase cellular suspension aliquots were re-fed with fresh RPMI-1640 medium two or three times per week. The stock solutions of the tested

compounds were freshly prepared in DMSO and consequently diluted in RPMI-1640. At the final dilutions obtained the concentration of the solvent never exceeded 0.5%.

Cell viability determination (MTT assay)

The MTT-dye reduction assay was carried out as described by Mosmann[10] with some modifications. Briefly, 100 µl aliquots of cell suspension (1 × 105 cells per ml) were seeded in 96-well microplates. Following 24 h incubation at 37 °C the cells were exposed either to the newly isolated lignan or toetoposide for 72 h. After the incubation period MTT solution (10 mg ml–1 in PBS) was added (10 µl per well) and the plates were further incubated for 4 h at 37 °C. Thereafter the formazan crystals formed were dissolved through addition of 100 µl per well 5% formic acid in 2-propanol (Merck) and the absorption of the samples was measured with an ELISA reader (Uniscan Titertec) at 570 nm. Hundred microliters of RPMI 1640 medium (Sigma), 10 µl MTT stock and 100 µl 5% formic acid in 2-propanol served as a blank solution. The results were expressed as survival fraction (% of untreated control).

CONCLUSION

In this report, quinoline derivatives were prepared by direct method from hydrochloric acid in dichloromethane and then was characterized by spectroscopic methods and X-ray crystallography. The cytotoxic effects of synthesized quinoline derivatives were tested against the leukemia derived Nalm6 cell lines The compound 5-chloro-8-hydroxyquinolinium chloride acts as better cytotoxicity system .

Supplementary Material

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-786214 .Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (Telefax: +1223/336 033; email: deposit@ccdc.cam.ac.uk).

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