New Synthesis of Chloroquinoline Derivatives as Anti- Cancer Agents

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Abstract
Autophagy is a cellular recycling system that degrades damaged or unneeded cellular organelles and proteins in a homeostatic manner. In the unfavorable metastatic environment, cancer cells are expected to utilise autophagy as a source of energy, and a number of clinical trials are now revealing the prospective role of chloroquine (CQ), an autophagy inhibitor, as a novel anticancer medication. CQ is frequently used in cancer treatment in conjunction with chemotherapeutic medicines and radiation. Chloroquine derivatives derived from 4, 7-dichloroquinoline and two anticancer medicines (doxorubicin and docetaxel) were synthesized and employed as anticancer agents in this study. The UV-Vis spectrophotometer, FTIR, CHN and HNMR were used to characterize chloroquine derivatives (CQ-DOX and CQ-DOC). Anticancer activity for each compound has been investigated and IC50 has recorded to be 2 µM and 0.715 µM respectively.
Keywords: chloroquine, anticancer, cancer treatment

1. Introduction

TNBC is a type of triple-negative breast cancer that is generally aggressive and has a bad prognosis. As a result of the absence of existing targeted therapies and problems with resistance to standard chemotherapeutic drugs, new treatments for TNBC become required [1].
Quinolines are aromatic compounds contained benzene ring fused to a pyridine heterocyclic system containing a nitrogen atom, they have a wide range of medical and pharmacological applications [2, 3].
In many malignant tumors, a number of drugs has ability to destroy autophagy progression at different stages, with different mechanisms, however, chloroquine (CQ) as an antimalarial medication qualified for therapeutic use, was developed to suppress autophagy and its linked upstream and downstream components [4, 5].
Chloroquine inhibits autophagic protein breakdown, which is the final stage in the autophagy mechanism, resulting in the accumulation of inefficient autophagosomes and the death of cells that rely on autophagy for life [6, 7]. Additionally, several clinical trials involving CQ and other chemotherapeutic medicines are now conducted [8].
The ability of CQ to inhibit autophagy in cancer cells and to normalize vessel function calls for a reconsidering of the therapeutic schedule and dosage of CQ-based therapies, as well as an assessment of potential side effects, in order to fully exploit the therapeutic potential of this well-established antimalarial agent in cancer therapy [8].
However, it is unclear whether chloroquine works entirely through autophagy-dependent or cancer cell-independent pathways [9].
It was reported that the autophagosome unites with lysosomes to produce an autolysosome, which allows various lysosomal hydrolytic enzymes to degrade the sequestered contents. Following degradation, amino acids, sugars, fatty acids, and nucleosides are produced, which are then recycled for macromolecular synthesis and energy production [10, 11]. During starvation, this recycling system is very critical. Autophagy is a cellular homeostasis mechanism that eliminates damaged proteins and organelles at low basal levels [10].
Chloroquine has been produced based on docetaxel and doxorubicin to improve chloroquine resistance and reveal anticancer medication adverse effects in this study.

2. Experimental Section

Chemical synthesis

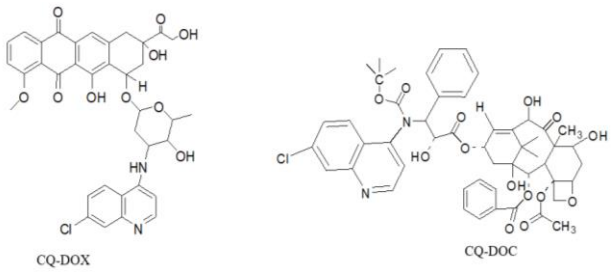

Figure1: The suggested structure of CQ-derivatives.

The synthesis of CQ-DOX

4, 7-dichloroquinoline (0.5 g, 0.0025 mol) and doxorubicin (0.0460g, 0.000084 mol) were heated at 120–130°C for 24 hours with stirring. After cooling, the reaction was poured into 100 mL of water and filtered, and the solid residue was dried before being heated in 100 mL of ethyl acetate to yield (o.6181 g, 81 %) as a reddish solid.13 Elemental analysis expected C: 62.75%, H: 4.83%, N: 4.07%: found C: 62. 09 %, H: 7.45 %, N: 4.12% for a chemical formula C36H33ClN2O10 with a molecular weight of 689.11.

The synthesis of CQ-DOC

4, 7-dichloroquinoline (0.5 g, 0.0025 mol) and docetaxel (0.08 g, 0.000098 mol) were heated at 120–130 °C for 24 hours with stirring. After cooling, the reaction was poured into 100 mL of water and filtered, and the solid residue was dried before being heated in 100 mL of ethyl acetate to yield (o.78g, 64%).13 Elemental analysis expected C: 64.11%, H: 5.80%, N: 2.93%: found C: 64. 46 %, H: 7.28 %,

doi.org/10.31838/hiv22.02.174 Received: 20.05.22, Revised: 21.05.22, Accepted: 12.08.22

N: 2.90% for a chemical formula C51H55ClN2O14 with a molecular weight of 955.44.
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2.1 Cell lines and cell culture

In Dulbecco's modified Eagle medium, cell line A-172 (ATCC.CRL-1620TM, brain, glioblastoma) was cultured (DMEM, ATCC.30-2002). 10 percent fetal bovine serum (FBS, GIBCO.26140079) and 100 units/mL penicillin-streptomycin were added to the cell line (Cellgro.30-002CI). Filter cap culture flasks with a surface area of 25 cm2 or 75 cm2 were used to cultivate the cells (Greiner Bio-One, Germany). Surface cells were trypsinated with 2 mL of 0.05 percent trypsin/0.53 mM EDTA in Hank's balanced salt solution when the confluency of adherent cells reached 80 percent of the flask (HBSS)

Application of drug and XTT cell viability assay

The target and control cell lines were A-172 glioblastoma breast cancer cell lines. A-172 cells were planted in 96 well plates (n = 5) with a 150 µL aliquot and left to develop overnight. After that, it was treated for 45 minutes with either CQ-DOX conjugate or CQ-DOC in a 10-fold dilution (10, 20,30,40,50 µL). The medium was removed after incubation, and each well was cleaned with 150 µL of washing buffer. After discarding the washing buffer, non-phenolic media was added to each well and cultured for 7 days before cell viability was determined. Cell viability was determined using an XTT-based in vitro toxicity assay kit. The medium was supplemented with an XTT solution containing PMSF at a concentration of 20% (v/v). At 450 nm, the absorbance was measured.

3. Results and Discussion

Chloroquine derivatives were created by combining dichloroquine (CQ) with doxorubicin (DOX) and docetaxel (DOC) to create novel CQ-DOX and CQ-DOC compounds that are more active than the original compounds.
Each conjugated chemical was identified using UV-Vis spectroscopy, with maximum wavelengths of 316, 325 nm for CQ-DOX and 319,325 nm for CQ-DOC, respectively.
FTIR spectra for DOX-CQ compound provided good evidence for the production compound, data displayed an intense band of stretching vibrations of N-H at 3350 cm-1 with no shoulder band which is indicated the conjugation between CQ and DOX. FTIR spectrum of the rest characteristic absorption peaks corresponding to vibrations of different functional groups of the drug molecule has been listed in Table1.

Table1: IR spectrum of CQ-DOX	
3084, 2862	**C–H stretch**
1635	C=O stretch
1593, 1558, 1491	C=C ring stretch
1197, 1087	C–O–C stretch
805, 688	C=H bend, C=C ring bend
1320	C-N-C

Similarly, FTIR spectra for CQ-DOC compound was indicated a clear extra band at 1350 cm-1 for stretching vibrations of C-N-C and disappearing a band at 3350 cm-1 for secondary amine of docetaxel which provided a good evidence for the conjugation between CQ and DOC. The rest of characteristic absorption peaks corresponding to vibrations of different functional groups of the molecule has been shown in Table2.

Table2: IR spectrum of CQ-DOC.	
2924, 2860	**C–H stretch**
1739,1647	C=O stretch
1560, 1504, 1456	C=C ring stretch
1356, 1109	C–O–C stretch
1350	C-N-C

On the other hand, the proton-NMR has been used for farther improvements for CQ-DOX and CQ-DOC production. 1HNMR spectra has been illustrated in Figures 2 and 3 respectively. The peak at 6.8 ppm due to secondary amine indicated the conjugation between CQ and DOX while, it has been absent in the proton NMR for CQ-DOC due to the conjugation of CQ with DOC.

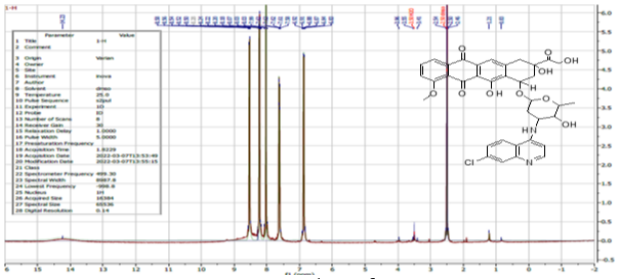

Figure 2: HNMR chart for CQ-DOX.

Anticancer activity

The activity of each CQ-derivative (CQ-DOX and CQ-DOC) was tested in vitro, and the results showed that each CQ-derivative has a significant inhibitory effect, whereas the same dose of chloroquine and each anticancer medicine DOX and DOC were applied to the target cell line A-172, the data represented that conjugated chloroquine (CQ-DOX and CQ-DOC) inhibited the proliferation of target cells A-172 more effectively in comparison to DOX and DOC. CQ, on the other hand, has no or just a minor activity Figure 5.

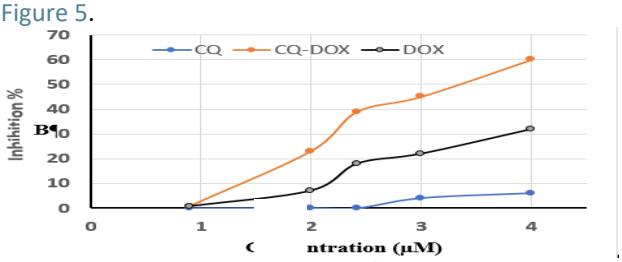

Figure 3: HNMR chart for CQ-DOC

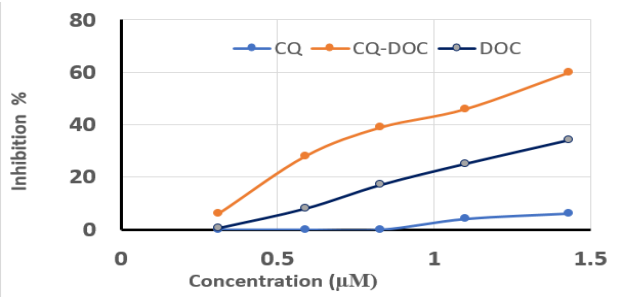

Figure 4: The effects of CQ-derivatives on A-172 cells inhibition in comparison with DOX, DOC and CQ alone.

Additionally, (IC50) was calculated for each derivative to be 2µM for CQ-DOX and 0.715µM for CQ-DOC respectively. Thus indicating the important role for each

compound to improve the activity of chloroquine as anticancer agent. Also the results showed that new synthesized chloroquine derivatives generated via conjugation with anticancer drugs are more active than a combination of anticancer pharmaceuticals.

4. Conclusion

Chloroquine is a potential cancer treatment that is also relatively safe, especially when used for a short time. Unexpected negative effects on organs, such as the kidney, may develop as a result of its inhibition of autophagy, especially when taken with anticancer medicines. As a result, practitioners in the fields of cancer and kidney therapy may be more aware of newly synthesized chloroquine derivatives that are formed from conjugation with anticancer medications rather than a combination, which improved chloroquine activity and might be reduced aware of clinician's therapy. Additionally, in-vivo studies on pharmacokinetics, toxicity and anti-tumor effects of these derivatives are required to develop their cytotoxic activity against tumor cells.

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