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Some Chloroquine Derivatives for Promising New Antifungal Drugs and Absorption Behavior

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Chloroquine (CQ) has been a good treatment for antimalarial mainstay for several decades; additionally discovered, it has a significant therapeutic impact on some instances of fungal inhibition. This study focused on the effect of novel CQ compounds on the activity of two different species of fungi, *Aspergillus niger* and Aspergillus falves. The activity of each CQ derivative was monitored using Nuclear magnetic resonance spectroscopy (NMR), minimal inhibitory concentration and physical parameters such as optical microscopy, UV/visible absorbance and optical band gap. NMR indicated the conjugation between the substrate and amino acid. The optical microscopic images indicated homogeneously distributed and uniform density distribution of CQ-derivative particles on the glass substrates. The samples' presented absorption peaks at 203, 207 and 220 nm wavelengths, suggesting the important electronic transition with reducing the indirect bandgap from 4.1 eV to 3.95 eV. These compounds have the best antifungal growth inhibitory properties and excellent features, indicating cosmetics use.

Keywords: Chloroquine; antifungal drugs; optical microscope; energy gap.

1. Introduction

Chloroquine (CQ) has a significant ability as an anisotropic agent and diffuses into acidic organelles that assist to use as a treatment or drug for several diseases,[1] and inhibits the growth of antibacterial or fungi such as antimalarial drug, antifungal capacity, *Candida albicans* and sensitizes biofilms of *Candida albicans* to antifungal azoles. Moreover, CQ directly impacts *Cryptococcus neoformans* and *Histoplasma capsulatum*, probably due to accumulating in the fungal vacuole.[2–4] Because CQ has a weak base and can increase the pH of eukaryotic

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cells' endocytic and lysosomes,[5,6] it diffuses into the acidic endosomes when it enters the phagocytic cell and could reach concentrations up to ten thousand times higher than in the extracellular levels.[7] The load of Chloroquine into the cells of human mononuclear has been recorded to have an additive impact on both *C. neoformans* as well as *H. capsulatum* death.[3,8] Moreover, CQ exhibits as a valuable drug to treat disorders, for instance, rheumatoid arthritis, which is linked to an increase in the release of pro-inflammatory cytokines, antagonizing TNF-expression produced by fungi (as *Candida albicans* and *C. neoformans*) and mononuclear phagocytes driven by lipopolysaccharide (LPS) through a mechanism involving endolysosome alkalization.[9–12]

Alkalinization of the fungi's host environment is suggested to be the process, with an iron shortage in some cases.[13] In yeast and human cells, CQ has been found to inhibit thiamine transport.[14] The availability of many fungal genome sequences has helped develop new antifungal drugs and elucidate mechanisms of action. Therefore, three CQ derivatives were synthesized and fully characterized before being tested as antifungal agents, and the physical properties of each compound have been investigated in this study.

2. Material and Methods

2.1. *Chemical synthesis*

All three CQ derivatives were synthesized in the previous study to produce CQ-ALA, CQ-P and CQ-VAL.[15]

2.2. *Agars culture media*

Potato dextrose agar medium (PDA) is fungus isolation and diagnosis medium. According to the manufacturer's instructions, the medium was made using 39 g of agar, which was dissolved in 1000 mL of distilled water, and sterilized in an autoclave for 20 min under controlling temperature at 121°C. It was cooling to 45°C, and adding 250 mg/L chloramphenicol.[16]

2.3. *Determination of in vitro antifungal activity*

The antifungal activity of each CQ derivative was determined using the agar disc diffusion method, which was conducted according to a published procedure.[17] The concentrations were made in (5 and 10) mg/mL and then deposited in 5 mm filtration paper discs that had previously been sterilized in an autoclave. To obtain 0.5 McFarland turbidity, isolated colonies from Sabouraud dextrose agar were suspended in 5 mL of 0.85 present sterile normal salines, resulting in a yeast stock solution of 1.5106 cells/mL. The dishes were left to dry after 0.1 mL and were moved to the surface of SDA, then published using swabbed (sterile cotton swabs) and dishes were left to dry. The filtration papers were spread to the surface of the cultured plates using sterile forceps at a rate of three tablets per dish and three duplicates per concentration, and then the dishes were incubated at 37°C for 72 h where the inhibition diameter was calculated for each concentration.[18]

2.4. *Minimum inhibitory concentration*

The concentration of minimum inhibitory was determined following the following procedure, where the agar dilution plate method was used with some modifications Wiegand *et al.*[19] Briefly, 1 mL from the highest concentration in two-fold dilution (5, 2.5, 1.25, 0.625) mg/mL of each CQ derivative was mixed with 10 mL of potato dextrose agar (PDA) and cooled to 50°C for control plates that did not contain CQ derivatives, 0.2 mL from *Aspergillus niger* and Aspergillus falves was added separately. In contrast, plates were left for 30 min and then incubated at 28°C for 48 h. The experiments were carried out in triplicate, and the lower concentration at the growth area was unavailable depending on the minimum inhibitory concentration. The results are reported based on the presence of growth (+) or absence of growth (−).[19]

2.5. *Physical properties*

The CQ derivatives' samples were first prepared for physical properties using the drop-casting method on a glass substrate. Then optical microscopic images at a magnification power of 100× and 40× and UV/visible characterizations were investigated using Nikon Olympus 73346 and Shimadzu 1800 spectrophotometers, respectively. All CQ derivatives samples showed significant homogenous diffusion on glass substrates with

no agglomerations, according to the results. The produced samples show a low energy band gap and high absorbance peaks in the UV region between 100 and 400 nm wavelength. These results make these samples promising materials for UV shielding and cosmetics.

3. Results and Discussion

Three CQ-derivatives had previously been synthesized and fully characterized; these derivatives' structure is shown in Fig. 1.

Proton NMR indicated each CQ compound according to NH-band, which identified the conjugation between the substrate (4,7-dichloroquine), and each amino acid, as shown in Fig. 2.

In this study, the morphological and optical characterizations, such as absorbance spectra and calculations of energy band gaps of each CQ-P, CQ-ALA and CQ-VAL, were investigated. Figure 3 illustrates the optical microscopic images that indicated a perfect activity of solubility and diffusion of compounds on the glass substrates. The particles of CQ-derivatives were homogeneously distributed on the glass surface substrates with uniform density distribution. The results also indicate that the CQ-derivative molecules tend to form well-dispersed groups and may indicate a homogeneous growth mechanism.[20]

Figure 4 shows the ultraviolet (UV) spectrums of CQ-P, CQ-ALA and CQ-VAL. The absorption peaks at 203, 207 and 220 nm wavelengths suggested the existence of an electronic transition from n to π^* produced by the chromophoric group

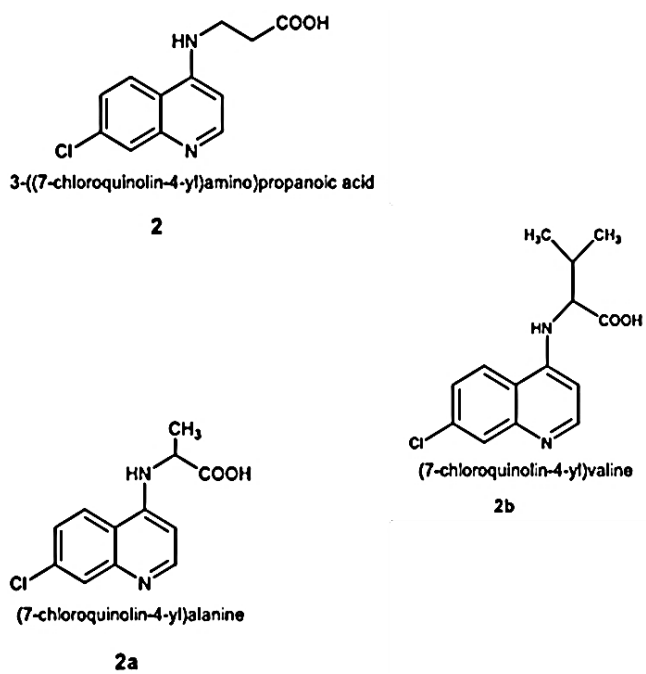

Fig. 1. The chemical structure of CQ-derivatives (2-CQP, 2a-CQ-ALA and 2b, CQ-VAL).

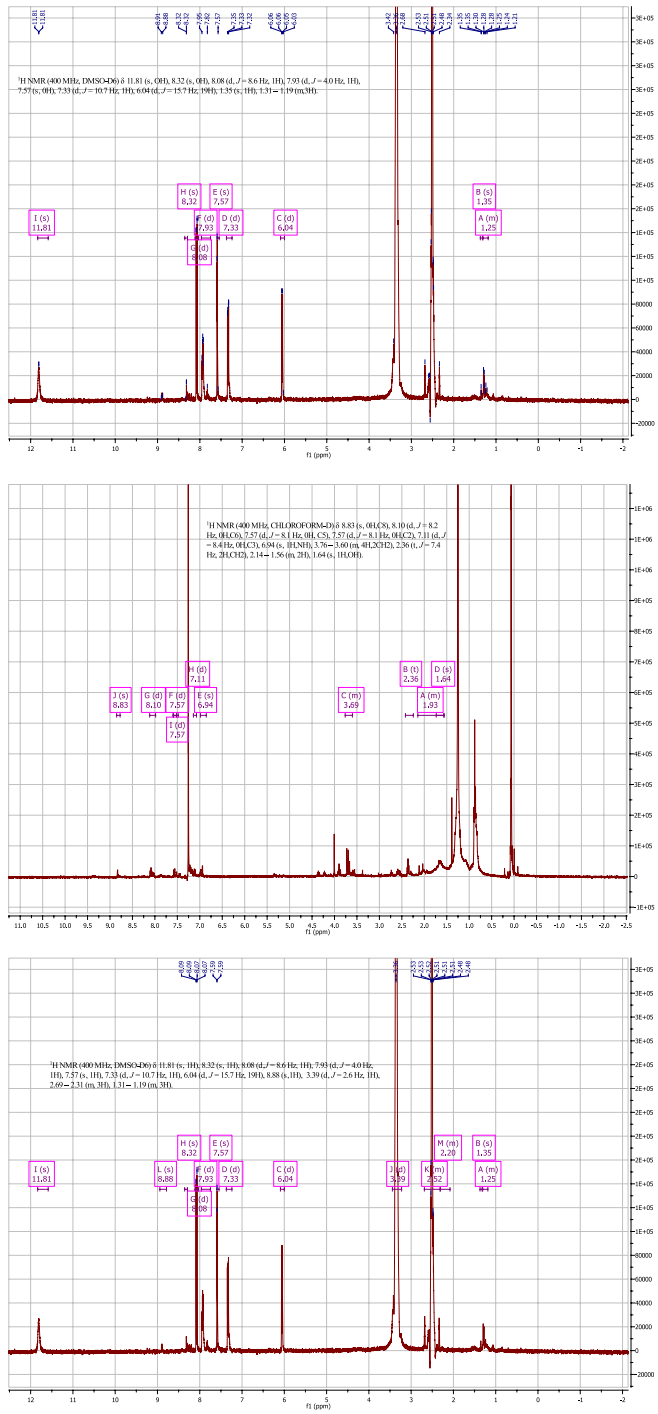

Fig. 2. The ^{1}HNMR chart for each CQ-derivative.

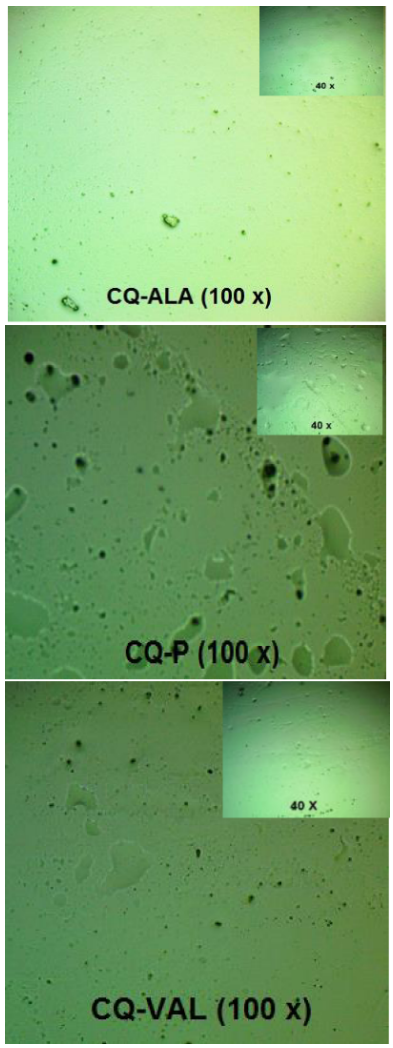

Fig. 3. The optical microscopic images of CQ-derivatives at the magnification powers of 100 and 40×.

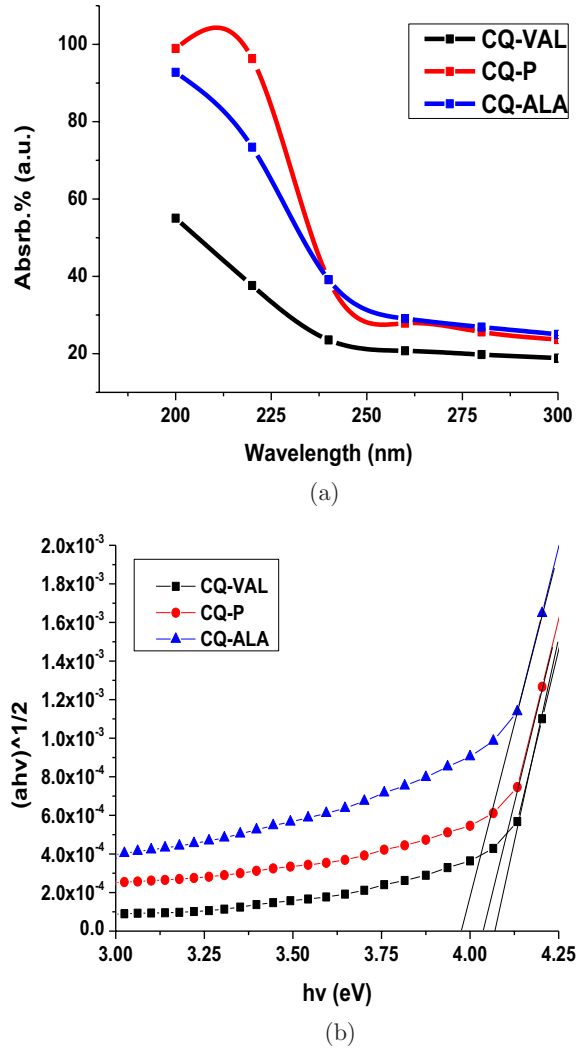

Fig. 4. The (a) optical absorbance and (b) energy band gap of CQ-derivatives.

of CQ-P, CQ-ALA and CQ-VAL.[21,22] These compounds have high optical absorbance intensities in the UV region (100–400 nm), making them suitable for various medical and optoelectronic applications, as shown in Fig. 4(a). Meanwhile, Fig. 4(b) shows a Tauc plot diagram $(\alpha h\upsilon)^{1/2}$ with energy $(h\upsilon)$. The optical energy gap of samples was calculated from the interception of the extrapolated linear component with the photon energy (E_g) applying the flowing equation (1):[23]

$$ahv = B(hv - E_g)^r, \tag{1}$$

where (hv) at $(\alpha hv)^{1/n} = 0$, (B) is constant, (h) is Planck's constant, (v) frequency, (E_g) and (r) exponential constant, which have various values depending on the transition types. For instance,

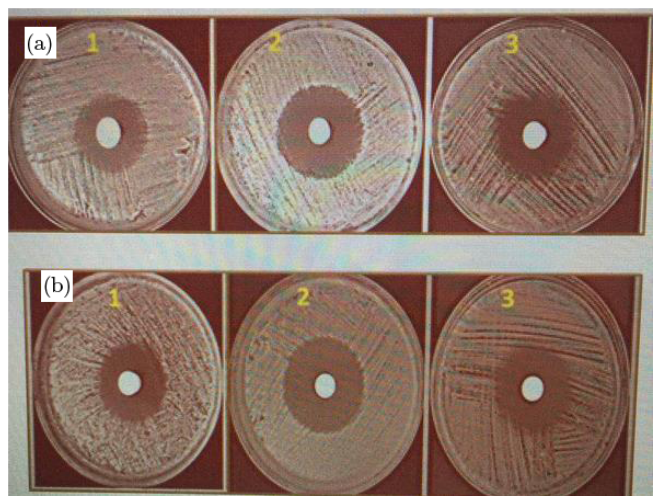

Fig. 5. Agar disk diffusion method after treatment with; 1: CQ-VAL, 2: CQ-ALA, CQ-P against (a) Aspergillus falves and (b) *Aspergillus niger*.

the (r) value is 1/2 and 3/2 for allowed and forbidden direct or 2 and 3 for indirect transitions, respectively.[24,25] The indirect energy band gap values are 4.1 eV, 4.05 eV and 3.95 eV for CQ-P, CQ-ALA and CQ-VAL, respectively. The values obtained are suitable for use in various potential electronic devices.[20,26,27]

Furthermore, the ability of each CQ-derivative as an anti-fungus agent has been tested against two species of fungi, namely (*Aspergillus niger* and Aspergillus falves). Against these two fungi, each CQ derivative (CQ-P, CQ-ALA and CQ-VAL) displayed a considerable inhibitory action. The inhibition zones, that is a circular area around the spot of each one in which the fungus colonies do not grow were measured using a ruler and found to be 21 cm, 23 cm and 20 cm for *Aspergillus niger*, and 20 cm, 22 cm and 18.5 cm for Aspergillus falves, respectively as presented in Fig. 5.

The MIC of these derivatives was determined to be 0.625 mg/mL, whereas when the concentration of both fungi was raised, the percentage of inhibition increased dramatically. The inhibition curves for CQ-derivatives in the presence of various concentrations of these two fungi are shown in Fig. 6. The results showed that each CQ-derivative interfered significantly with fungus activity *in vitro*. Because CQ-ALA has direct toxicity by elevating the phagolysosomal pH that restricts the intracellular infections growth, it has demonstrated the best effectiveness against Aspergillus species.

The minimum percentage of fungal growth (Emin) detected in each derivative was higher for *Aspergillus niger* (32, 50 and 28%) than another *Aspergillus* spp. (10, 30 and 20%), respectively.

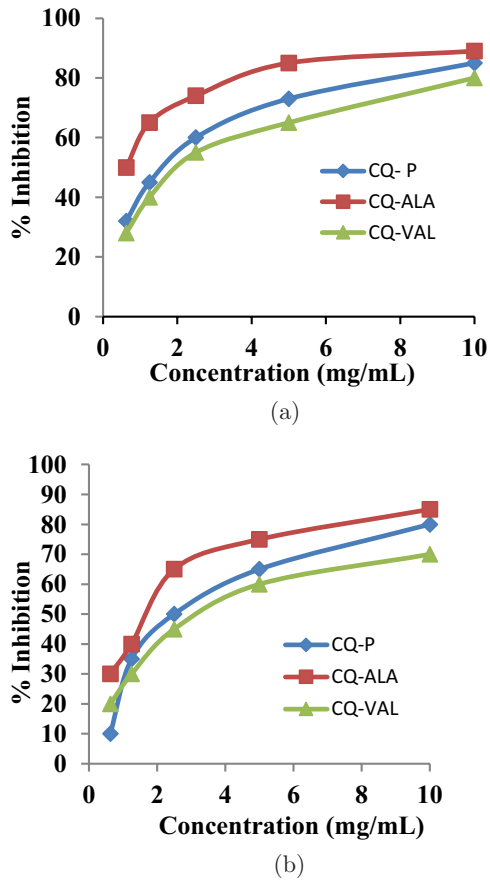

Fig. 6. Concentration effect curves of CQ-derivatives against (a) *Aspergillus niger* and (b) Aspergillus falves.

4. Conclusion

The inhibitory effects of CQ-derivatives on both Aspergillus species were excellent at high concentrations. The free drug hypothesis may directly apply *in vitro* for antifungal medicines, implying that unique processes may be involved *in vitro* activity. CQ-ALA has the best inhibitory impact on both species compared to the other two CQ-derivatives. This may be used for the CQ side chain's participation in fungus cell death.

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