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ORIGINAL RESEARCH PAPER

NEW QUINOLONE-BASED COMPOUNDS: SYNTHESIS, CRYSTAL STRUCTURES AND BIOLOGICAL ACTIVITIES

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Abstract: A new quinoline-based compounds have been synthesized from 2-oxo-1,2-dihydroquinoline-4-carboxylic acid (2) and involved in alkylation reactions using phase transfer catalysis conditions. The esterification and protection of the carboxylic acid function of (2) have been also studied. The structures of the prepared compounds have been determined by spectroscopic methods: IR, ^{1}H NMR, ^{13}C NMR and the structure of compound 4 was confirmed by single crystal X-ray diffraction. The antioxidant activity of these compounds was evaluated using DPPH, FRAP and *β*-carotene bleaching techniques. Their antimicrobial activity was evaluated against *E. coli, S. aureus, B. subtilis, B. cereus* and *Salmonella typhi* using a microplate protocol.

Keywords: *alkylation, antioxidant and anti-bacterial activities, quinolone, X-ray diffraction*

INTRODUCTION

Quinolone derivatives are considered as useful compounds in medicinal chemistry. Some natural quinolone derivatives have shown a pharmacological profile with an exceptional antibacterial activity due to their excellent bioavailability and ability to enter macrophages [1 – 4]. Among the most interesting quinolone nuclei are those acting as anti-tuberculosis and antibacterial agents [5] such as fluoroquinolones. They also possess remarkable biological activities such as anti-tumoral and antimicrobial against the agents of respiratory nosocomial, gastrointestinal, urinary tract, skin and soft tissue infections, chronic osteomyelitis and sexually transmitted diseases [6 – 10].
Also some quinolones presented a confirmed bactericidal activity on sensitive strains [11]. Their applications in various fields such as: herbicides, dyes, veterinary medicine and as agrochemicals have also been reported [12]. Similarly several quinolone derivatives are used in the treatment of malaria [13, 14], cardiovascular disease [15] and as antifungal [16], antitumoral [17] and antimicrobial agents against pathogenic microorganisms, especially resistant bacteria [18, 19].
In this work we are interested in the synthesis of new heterocyclic systems containing quinolone moiety in the aim of evaluating their biological activities on studying their antibacterial activity by determining the minimum inhibitory concentration and bactericidal effect, on the one hand, and the antioxidant power using DPPH, FRAP and *β*-carotene bleaching activity was also examined.

MATERIALS AND METHODS

Chemistry

Melting points were taken using Electrothermal (9100). Column chromatography was performed on silica gel 60 (Merck 230-400 mesh). NMR spectra were recorded on Bruker device types AC-300. Chemical shifts are given in parts per million (ppm) and DMSO or $CDCl_3$ were used as solvents. All solvents and reagents purchased from commercial suppliers were used without further purification. Key: s (singlet), d (doublet), t (triplet), m (multiplet). The FT-IR spectrum of samples was recorded on VERTEX 70 BRUKER TGA- IR.

Synthesis of *2-oxo-1,2-dihydroquinoline-4-carboxylic Acid* 2

To a solution of isatin (10 mmol) in 30 mL of acetic acid were added malonic acid (10 mmol) and sodium acetate (10 mmol). The reaction mixture was refluxed for 24 hours. After cooling 100 mL of water ice were added. The precipitate obtained was washed several times with ethanol. The product was recrystallized from water.
Yield (%)= 90 %; Mp: >300 °C; ^{1}H NMR (300 MHz, DMSO-d6): 6.86 (s,1H, CH); 7.2-8.16 (m, 4H, CH_{arom}); 12.17(s, 1H, NH); 13.9 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d6): 167.2 (COOH), 161.4 (C=O), 141.7-139.8 (Cq, Cq), 131.3 (CH_{arom}), 126.5 (CH_{arom}), 123.8 (CH), 122.6 (Cq), 116.2 (CH_{arom}).

Synthesis of *ethyl 2-oxo-1,2-dihydroquinoline-4-carboxylate* 3

To a solution of compound 2 (1.9 g, 10.0 mmol) in 20 mL of absolute ethanol, 1 mL of sulfuric acid was added. The reaction mixture was kept overnight at reflux, then the ethanol was evaporated under reduced pressure and the residue was treated with DCM and water (20 mL) with subsequent evaporation. This process was repeated three times to remove H_2SO_4. The pure compound was obtained by recrystallization from H_2O.
Yield (%) = 94 %; Mp: 252 °C ^{1}H NMR (300 MHz, $CDCl_3$): 1.35 (t, 3H, $^3J_{H-H}$ = 7.2 Hz, CH_3); 4.4 (q, 2H, $^3J_{H-H}$ = 7.2 Hz, CH_2); 6.89 (s, 1H, CH); 7.24 (m, 1H, CH_{arom}), 7.39 (d, 1H, $^3J_{H-H}$ = 7.8 Hz, CH_{arom}); 7.6 (m, 1H, CH_{arom}); 8.04 (m, 1H, CH_{arom}); 12.14 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): 165.51 (COOH), 161.27 (C=O), 140.67-139.87 (Cq, Cq), 131.55 (CH_{arom}), 126.26 (CH_{arom}), 124.28 (CH_{arom}) 122.85 (CH), 116.32 (CH_{arom}), 115.89 (Cq),62.36(CH_2), 14.38(CH_3).

Synthesis of *ethyl 2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylate* 4

To a solution of compound 3 (1g 4.6 mmol) in 10 mL of DMF were added (0.94 g,1.5 mmol) of propargylic bromide, (1.46 g, 2 mmol) of K_2CO_3 and (0.07g, 0.1 mmol) of tetra *n*-butylammonium bromide (TBAB). The reaction mixture was stirred at room temperature in DMF for 6 hours. After removal of salts by filtration, the DMF was evaporated under reduced pressure and the residue obtained was dissolved in dichloromethane. The organic phase was dried over Na_2SO_4 then concentrated in vacuo. Pure compound was obtained after recrystallization from dichloromethane / hexane (1/3).
Yield (%) = 82 % ; Mp: 126 °C ^{1}H NMR (300 MHz, $CDCl_3$): 1.44(t, 3H, $^3J_{H-H}$ = 7.2 Hz, CH_3) 2.88 (t, 1H, $^3J_{H-H}$ = 2.4Hz, ≡CH); 4.48 (q, 2H, $^3J_{H-H}$ = 7.2 Hz, CH_2-O); 5.14(d, 2H, $^3J_{H-H}$ = 2.4 Hz CH_2-N); 7.21 (s, 1H,CH); 7.34 (m, 1H, CH_{arom}), 7.6 (d, 1H, $^3J_{H-H}$ = 8.1 Hz, CH_{arom}); 7.68 (m, 1H, CH_{arom}); 8.38 (dd, 1H, $^3J_{H-H}$ = 9.3Hz, $^4J_{H-H}$ = 1.2Hz CH_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): 165.15 (COOH), 160.50 (C=O), 139.68-139.01 (Cq, Cq), 131.28 (CH_{arom}), 127.38 (CH_{arom}), 123.79 (CH_{arom}) 123.09 (CH), 117.78 (Cq), 115.01 (CH_{arom}), 72.76 (C≡CH), 62.13 (CH_2), 31.92(CH_2) , 14.15(CH_3).

Synthesis of *pyridin-1-ium 2-oxo-1,2-dihydroquinoline-4-carboxylate* 5

A solution of 10 mL of compound 2 (1g, 5.3 mmol) in pyridine (10 mL) was heated at 100 °C for 2h. After complete conversion the solvent was evaporated under reduced pressure and the residue obtained was washed with diethyl ether to give a solid gray compound.
YF 5 Yield (%) = 80 %; ^{1}H NMR (300 MHz, $CDCl_3$): 6.87 (s, 1H, , CH); 7.22 (t, 1H, $^3J_{H-H}$ = 7.5 Hz, CH_{arom}); 7.38 (m, 3H, CH_{arom}); 7.54 (t, 1H, $^3J_{H-H}$ = 7.5 Hz, CH_{arom}); 7.78 (t, 1H, $^3J_{H-H}$ = 7.5 Hz, CH_2); 8.16 (d, 1H, $^3J_{H-H}$ = 8.1 Hz, CH_{arom}); 8.57 (d, 1H, $^4J_{H-H}$ = 4.2 Hz, CH_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): 167.30 (COOH), 161.52 (C=O), 149.97 (2 CH_{arom}), 141.86-139.90 (Cq-Cq), 136.67 (CH_{arom}), 131.27 (CH_{arom}), 126.61 (CH_{arom}), 124.38 (CH_{arom}), 123.76 (CH_{arom}), 122.27 (CH_{arom}), 116.27 (CH_{arom}), 116.22 (Cq).

Synthesis of *2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylic acid* 6

Method 1 **(5 to 6):**To a solution of compound **5** (1g, 3.7 mmol) in 10 mL of DMF were added (0.66 g, 1.5 mmol) of propargyl bromide, (1.02 g, 2 mmol) of K_2CO_3 and (0.05g, 0.1 mmol) of TBAB. The reaction mixture was stirred at room temperature in DMF for 6 hours. After removal of salts, the solvent was evaporated under reduced pressure and the residue obtained was dissolved in dichloromethane. The organic phase was dried over Na_2SO_4 then concentrated in vacuo. Pure compound was obtained after precipitation in a mixture of dichloromethane/hexane (1/3) with 75 % of yield.

Method 2 **(4 to 6):** The product **4** (0.5g, 1.9 mmol) was saponified in a mixture of THF / EtOH / H_2O (8/8/8 mL) with NaOH (0.47g, 11.75 mmol) at 80 °C for 1 hour. The solvent was evaporated under reduced pressure. The crude obtained was taken up with 10 mL of water and citric acid is added until *p*H=4. The precipitate was filtered, washed with EtOH and diethyl ether and dried (Yield = 87 %).

Method 3 **(7 to 6):** The product **7** (0.5g, 1.88 mmol)) was saponified in a mixture of THF / EtOH / H_2O (8/8/8 mL) with NaOH (0.45g, 11.25 mmol) at 80 °C for 1 hour. The solvent was concentrated in vacuo. The crude was taken up with 10 mL of water and citric acid is added until *p*H=4. The precipitate was filtered, washed with EtOH and diethyl ether and dried.
YF 6 Yield = 91 %; Mp: 290 °C ^{1}H NMR (300 MHz, $CDCl_3$): 2.08 (s, 1H, C≡CH); 5.28 (s, 2H, CH_2); 6.95 (s, 1H, CH); 7.32 (t, 1H, $^3J_{H\text{-}H}$= 7.2 Hz, CH_{arom}); 7.40 (d, 1H, $^3J_{H\text{-}H}$= 8.4Hz, CH_{arom}); 7.61 (t, 1H, $^3J_{H\text{-}H}$= 7.5Hz, CH_{arom}); 8.16 (d, 1H, $^3J_{H\text{-}H}$= 8.1Hz, CH_{arom});^{13}C NMR (75 MHz, $CDCl_3$): 167.21 (COOH), 160.70 (*C*=O), 140.17-138.5 (Cq-Cq), 131.74 (CH_{arom}), 127.22 (CH_{arom}), 122.93 (CH_{arom}) 121.83 (CH_{arom}), 117.04 (Cq) , 115.75 (CH_{arom}), 52.22(CH_2), 27.83 (C≡CH).

Synthesis of *prop-2-yn-1-yl 2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylate* 7

To a solution of compound 2 (1 g, 5.63 mmol) in 10 mL of DMF were added (1.04 mL,11.5 mmol) of propargyl bromide, (2.19 g, 15.84 mmol) of K_2CO_3 and (0.17g, 0.5 mmol) of TBAB. The reaction mixture was stirred at room temperature in DMF for 6 hours. After removal of salts, the solvent was evaporated under reduced pressure and the residue obtained was dissolved in dichloromethane. The organic phase was dried over Na_2SO_4 then concentrated in vacuo. Pure compound was obtained after recrystallization from dichloromethane/hexane (1/3).
YF 7 Yield (%) = 89 % ; Mp: 162 °C ^{1}H NMR (300 MHz, $CDCl_3$) : 2.88 (t, 1H, $^3J_{H\text{-}H}$= 5.1Hz, $^4J_{H\text{-}H}$= 2.4Hz, C≡CH); 2.59 (t, 1H, $^3J_{H\text{-}H}$= 5.1Hz, $^4J_{H\text{-}H}$= 2.4Hz, C≡CH); 5.0 (d, 2H, $^4J_{H\text{-}H}$= 2.4 Hz, CH_2); 5.1(d, 2H, $^4J_{H\text{-}H}$= 2.4 Hz CH_2); 7.32 (s, 1H,CH); 7.34 (m, 1H,CH_{arom}), 7.6 (d, 1H, $^3J_{H\text{-}H}$= 8.4 Hz, CH_{arom}); 7.68 (m, CH_{arom}); 8.38 (dd, 1H, $^3J_{H\text{-}H}$= 8.1Hz, $^4J_{H\text{-}H}$= 1.2Hz CH_{arom});^{13}C NMR (75 MHz, $CDCl_3$): 164.32 (COOH), 160.25 (*C*=O), 139.04-138.5 (Cq-Cq), 131.41 (CH_{arom}), 127.26 (CH_{arom}), 124.49 (CH_{arom}) 123.18 (CH), 117.55 (Cq) , 115.78 (CH_{arom}),75.89 (C≡CH), 72.82 (C≡CH), 53.31(CH_2), 31.97(CH_2).

Study of Antioxidant Properties

Free radical scavenging activity

The free radical scavenging activity of our compounds was evaluated according to the method proposed by Jeong et al. [20] with some modifications. Briefly, 1 mL of compounds (**2**-**7**), in various concentrations, was mixed with 0.9 mL of compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.041 mM). Both DPPH and compounds (**2**-**7**)were dissolved in ethanol. Butylated hydroxytoluene (BHT) solution was used as a standard. After 10 min of incubation, the absorbance of the mixture was read at 517 nm against a blank. The following equation was used to calculate the inhibitory percentage of **Radical Scavenging Activity:**

$$(RSA) = \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] \times 100$$

IC50 value ($mg \cdot mL^{-1}$) is the concentration at which the scavenging activity was 50 % and was calculated from the graph of inhibition percentage against extract concentration**.**

Reducing power

The reducing power was determined according to the method of Bougandoura et al. [21]. Various mixtures of 1 mL of our compounds (2-7) (1-10 $mg \cdot mL^{-1}$), phosphate buffer (2.25 mL, 0.2 M, *p*H 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.25 mL, 1 %) were prepared and incubated at 50 °C for 20 min. 2.25 mL of trichloroacetic acid (10 %) were added to the mixtures which were then centrifuged at 3000 rpm for 10 min. The upper layer of the solutions (2.25 mL) were mixed with distilled water (2.25 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance of the reaction mixtures at 700 nm indicated increased reducing power. BHT was used as a positive control.

Inhibition of *β*-carotene bleaching

Lipid peroxidation inhibition (LPI) activity was evaluated according to the β-carotene bleaching method of Boudjou et al. [22]. 1 mL of *β*-carotene (0.2 $mg \cdot mL^{-1}$) dissolved in chloroform was mixed with linoleic acid (40 µL) and Tween-80 (400 µL). The solvent was removed and 100 mL of demineralized water was added. 5 mL of this mixture was added to 0.5 mL of every one of our compounds (1 $mg \cdot mL^{-1}$). EtOH 80% was used as control or BHT as standard. The absorbance was measured periodically after 15 min, against a blank at 470 nm, until the end of the experiment (t = 120 min). LPI activity was expressed as AOA (%) and was calculated as follows: The method of calculation was based on first order kinetics as described by Al-Saikhan et al. [23] using the following equations **bleaching rate of *β*-carotene**:

$$(BR) = ln\left(\frac{A_0}{A_t}\right) \times \frac{1}{t}$$

where A_0 is the initial absorbance (470 nm) of the emulsion at time 0, while A_t its the absorbance (470 nm) at 15, 30… and 120 min; and t is the time in min. The rates for

different times were averaged to give one value for the sample. The antioxidant activity was expressed based on the oxidation rate ratio, ORR, which was calculated by the method of Marinova et al. [24] using the equation:

$$(ORR) - \left(\frac{BR_s}{BR_0}\right)$$

BR_s is the rate of β-carotene bleaching in the presence of the sample, and BR_o is the rate of bleaching in the absence of the sample (blank). The antioxidant activity (AOA %) was calculated as % inhibition relative to the control using the following relationship [25]:

$$(AOA\%) = \left[1 - \left(\frac{BR_{sample}}{BR_{control}}\right)\right] \times 100$$

Antibacterial activity evaluation

Determination of Minimal Inhibitory Concentration (MIC)

The determination of MIC was performed in a 96-well microplate using the micro-dilution assay according to the protocol previously described by Bouhdid et al. [26] with slight modifications. Firstly, the product was serially diluted in DMSO, used as an emulsifier. Then, 50 µL of bacterial inoculum were added to each well at a final concentration of 10^6 CFU·mL^{-1}. The 12th was considered well as growth control. After incubation at 37 °C for 18-20 hours, 10 µL of resazurin were added to each well as bacterial growth indicators [27].

After further incubation at 37 °C for 2 h, the bacterial growth was revealed by the resazurin coloration changing from purple to pink. The MIC value was determined as the lowest concentration that prevented a change in resazurin color. Experiments were carried out in triplicate.

Determination of Minimal Bactericidal Concentration (MBC)

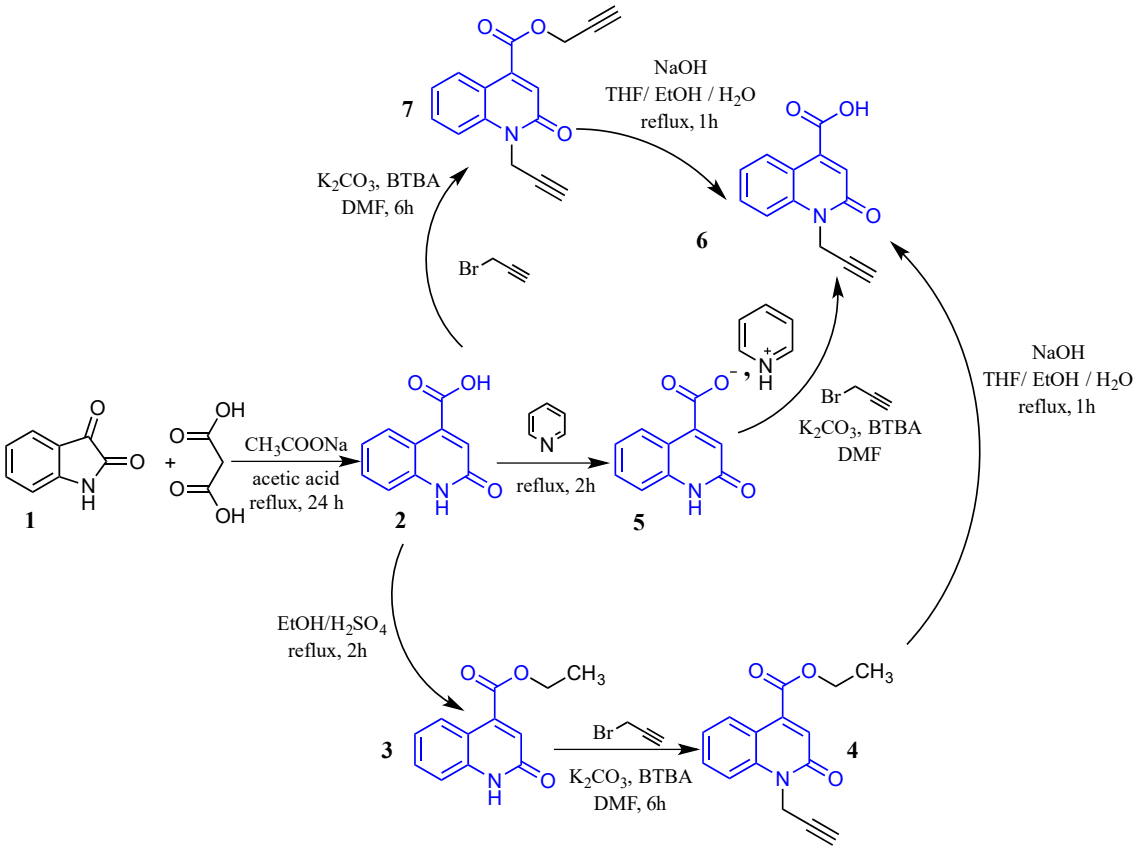
The MBC value corresponds to the lowest concentration of compounds yielding negative subcultures after incubation at 37 °C for 24 h. It was determined by spreading 5 µL from negative wells on Luria Bertani (LB) plates. Experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Chemistry

The starting material namely 2-oxo-1,2-dihydroquinoline-4-carboxylic acid (2) was performed by condensation of isatin with malonic acid in refluxing acetic acid. The carboxylic group was converted to an ethyl ester group by refluxing (2) in absolute ethanol with H_2SO_4 as a catalyst to form ethyl 2-oxo-1,2-dihydroquinoline-4-carboxylate compound (3) which is a white solid obtained in a yield of 94 %. In the last step, compound (3) was reacted with propargyl bromide under phase transfer catalysis conditions using K_2CO_3 as base and TBAB as a catalyst in DMF at room temperature.

Yellow crystals of (4) were obtained, in a yield of 82 % after recrystallization from DCM/hexane. The product (2) was also reacted with two alkylating agents (ethyl iodide and propargyl bromide) in the presence of TBAB and using K_2CO_3 as a base in DMF to give two different new compounds respectively prop-2-yn-1-yl 2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylate (7) in a yield of 79 % obtained after recrystallization from DCM/EtOH. Therefore, treatment of acid function of product (2) with pyridine gave the salt pyridin-1-ium 2-oxo-1,2-dihydroquinoline-4-carboxylate (5) in 93 % yield. Then compound (5) was immediately reacted with propargyl bromide under PTC conditions in DMF to give 2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylic acid in yield (6) after washing by H_2O. In a different way, the product (6) was obtained in a 91 % yield by a saponification reaction of compound (4) (NaOH, in mixture of THF, EtOH and H_2O for 1 h in reflux) and compound (7) in a 90 % yield (Scheme 1).



Scheme 1. *Synthesis of 2-oxo-1,2-dihydroquinolin-2-one derivatives*

Characterization of compounds prepared (2-7)

The structures of all synthesized compounds were assigned by IR, ^{1}H NMR and ^{13}C NMR spectral analysis. The IR spectra highlight absorption bands of deformation and stretching vibrations of compound (2). The presence of carboxylic acid (**CO_2H**) group was confirmed by the absorption bands at 2250-3300 cm^{-1} (ν **OH**: stretching vibration of hydrogen bonded OH groups) and 1715 cm^{-1} (ν **C=O** bonded). The lactam (amide) was identified by the presence of absorption bands at 1649 cm^{-1} (ν **C=O**), 1431 cm^{-1}

(δ **N-H**), 1229-1275 cm^{-1} (δ **N-H** bending coupled with ν =**C_{ar}-N**), 876 cm^{-1} (δ_{oop} **N-H**), 709 and 652 cm^{-1} (**O=C-N** bending) [28, 29]. The presence of ethoxycarbonyl group (**CO_2Et**) in product **(4)** has been confirmed by an absorption band at 1730 cm^{-1}. The N-propargylic alkylation was confirmed by the presence of an absorption band of **≡C-H** alkyne at 3300 cm^{-1} [30].

The ^{1}H NMR spectra of compound (2) showed a slightly broadened signal for the **(NH)** at δ 12.17ppm, and a singlet for the carboxylic acid **(CO_2H)** is observed at δ 13.9 ppm. The usual signals due to the quinolone moiety appear at δ 6.86-8.19 ppm. The ^{13}C NMR spectrum of (2), exhibited two signals at 167.2, 161.4ppm related to the carboxylic and lactamic groups respectively and five signals at 131.3, 126.5, 123.8, 122.6, 116.2 ppm corresponding to quinolone ring. In the ^{1}H NMR spectrum of compound (3) we note the presence of the characteristic signals of the ethyl ester group (triplet at δ 1.44 ppm **(CH_3)** and quadruplet at δ 4.48 ppm **(O-CH_2)**). The ^{13}C NMR of compound (3) showed the presence of 12 carbons and also a signal at 165.51 ppm attributed to the ethoxycarbonyl group **(CO_2Et)** and other signals at 161.27ppm (**C=O,** lactam), 62.36 ppm**(CH_2)**, 14.38 ppm**(CH_3)**. The ^{1}H NMR spectrum of compound (4) highlights the presence of the signals due to the N-propargylic group which appear at δ 2.88 ppm **(≡CH)** and δ 5.14 ppm **(N-CH_2)**. The ^{1}H NMR spectra of compound (5) showed the presence of two fragments, the quinolone and the pyridine that appears between 6.87ppm and 8.57 ppm; and the ^{13}C NMR spectrum of compound (5) confirmed the presence of all aromatic carbons at 149.97 (**2 CH_{arom}**), 136.67 (**CH_{arom}**), 131.27 (**CH_{arom}**), 126.61 (**CH_{arom}**), 124.38 (**CH_{arom}**), 123.76 (**CH_{arom}**), 122.27 (**CH_{arom}**), 116.27 (**CH_{arom}**). The ^{1}H NMR spectra of compound (6) also confirmed the presence of the N-propargylic group, **≡C-H** (signalat δ 2.08 ppm) and **CH_2-C≡** (signalat 5.28 ppm). The ^{13}C NMR spectra show a signal at 52.22 ppm attributed to the (N-**CH_2**) group and another signal at 27.83 ppm related to the (-C≡**CH group**).

It is worthy to note that the structures of compounds (2) [31], and (4) were confirmed by single crystal X-ray diffraction (Figures 1 and 2).

X-ray Crystallography

XRD data were collected at 150 K on a Bruker D8 Venture diffractometer equipped with a Cu microsource (CuKα, λ = 1.54178 Å) and a Photon 100 CMOS detector under control of the *APEX2* software [32]. Data processing was performed with *SAINT* and *SADABS* [32], the structure solved by direct methods (*SHELXT* [33]) and refined by full-matrix, least-squares methods (*SHELXL 2014/7* [34]). The alkynyl hydrogen atom was refined while all other hydrogen atoms were included as riding contributions in calculated positions.

Crystal structure of *ethyl 2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinoline-4-carboxylate* 4

A colorless parallelepiped-like specimen of $C_{15}H_{13}NO_3$, approximate dimensions 0.096 mm x 0.205 mm x 0.358 mm, was used for the X-ray crystallography analysis. The Crystal data, data collection and structure refinement details are summarized in Tables 1 and 2. Figures 1 and 2 show the molecular structure and packing respectively of **(4)**.

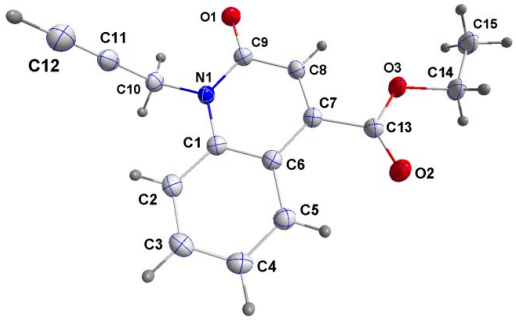


Figure 1. *Crystal structure of compound* ***(4)***

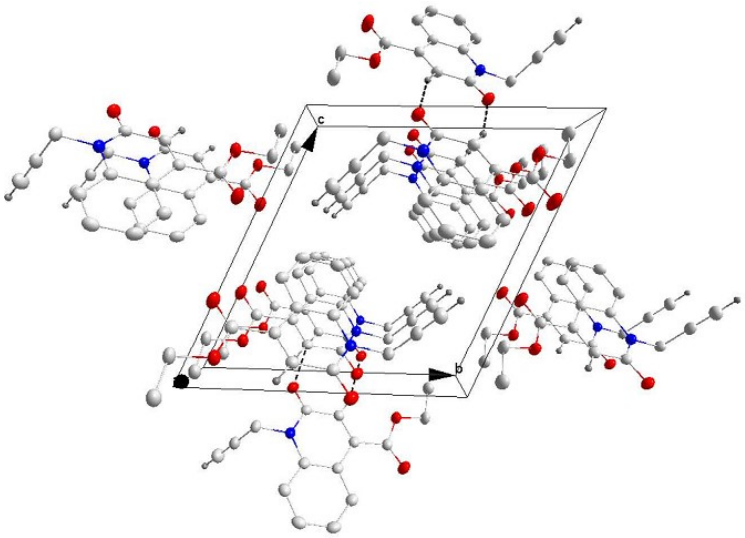


Figure 2. *Molecular packing of compound* ***(4)***

Table 1. *Sample and crystal data for* ***(4)***

Chemical formula	$C_{15}H_{13}NO_3$	
Formula weight	255.26 g·mol^{-1}	
Temperature	150(2) K	
Wavelength	1.54178 Å	
Crystal size	0.096 x 0.205 x 0.358 mm	
Crystal habit	colorless parallelepiped	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 4.71240(10) Å	α = 65.8180(10)°
	b = 11.5482(3) Å	β = 88.8610(10)°
	c = 12.2917(3) Å	γ = 86.5720(10)°
Volume	609.11(3) Å^3	
Z	2	
Density (calculated)	1.392 g·cm^{-3}	
Absorption coefficient	0.802 mm^{-1}	
F(000)	268	

The X-ray crystallography coordinates for structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under deposition numbers CCDC 1833338.

Biological activities

Study of Antioxidant activities

The antioxidant activity of our compounds was evaluated by three different methods: radical scavenging power, *β*-carotene bleaching and ferric reducing antioxidant capacity. DPPH and FRAP test results were expressed as BHT (butylated hydroxyl toluene) equivalent per one gram of our compounds (mg BHT eqv·mg^{-1}). The data showed that the radical scavenging power and ferric reducing antioxidant capacity were concentration-dependent (Table 2) because the rates of reduced DPPH and Fe^{3+} increases with the rise of the concentration of our compounds.

Table 2. *Antioxidant activity of compounds*

Products	**DPPH**		**FRAP**	***β*-carotene bleaching**
	IC_{50} [mg·mL^{-1}]	IC_{50} [mg BHT eqv·mg^{-1}]	[mg BHT eqv·mg^{-1}]	AOA [%]
Product (4)	5±0.6	0.0065	0.013	42.64±1.03
Product (3)	4±0.1	0.0081	0.065	49.11±0.11
Product (2)	3.7±0.05	0.0087	0.068	51.01±0.21

Radical trapping by DPPH is widely used to evaluate the antioxidant activity of different compounds. The DPPH reduction was followed by absorption measurement at 517 nm. It loses this adsorption when accepting an electron or free radicals which results in a distinct color change from purple to yellow [35]. The IC50 obtained using our compounds were much higher than the BHT standard. In addition, the data showed that the ferric reducing antioxidant capacity was much higher than the radical scavenging activity, 0.013 mg BHT eqv·mg^{-1} and 0.0065 mg BHT eqv·mg^{-1} respectively for the product **(4)**. Also it was the same for the **(3)** which showed a higher antioxidant activity compared to compound **(4)**. The radical scavenging activity of compound **(3)** showed an IC_{50} equal to 0.0081± mg BHT eqv·mg^{-1}. The reducing power of **(3)** was also stronger than the one of compound **(4)**. The antioxidant activity of **(2)** was the best concerning its radical scavenging form and the reducing power. From the Table 3 and according to the Figure 1, the synthesis procedure has a lower effect on the antioxidant activity. The antioxidant activity of the last product **(4)** is clearly lower than that of the compound **(2)**. All the other compounds were unable to reach the IC50 at the radical scavenging activity and ferric reducing power. *β*-carotene is attacked by peroxyl radicals that result from thermal-induced oxidation of linoleic acid. The antioxidant power of **(4)** attempts to reduce bleaching by stabilizing the peroxyl radicals, thus showing an AOA % of 42.64±1.03 %. On the other hand, compounds **(2)** and **(3)** were more effective in the peroxyl radicals' stabilization. The *β*-carotene bleaching test showed that compound **(2)** was the best preserving 51.01±0.21 % of *β*-carotene, and the AOA % for the **(3)** was 49.11±0.11 %. These values (51.01±0.21 % and 49.11±0.11 %)

show that compounds **(2)** and **(3)** have the same antioxidant activity. Compounds **(5)**, **(6)**, and **(7)** showed an AOA % of 16.57 %, 18.60 % and 21.70 % respectively.
The figure 3 shows the kinetic of *β*-carotene bleaching in the presence of our molecules which was monitored by following the *β*-carotene absorption at 470 nm.

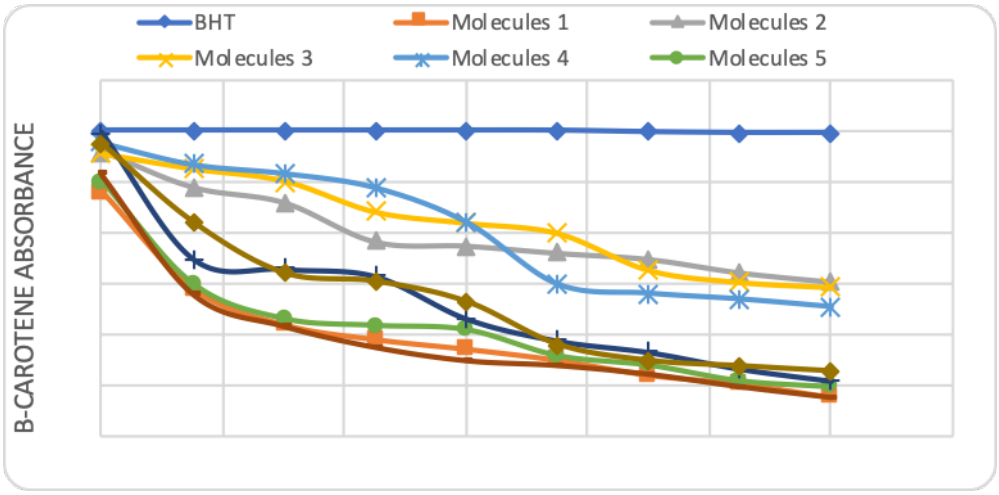


Figure 3. *The β-carotene kinetic blanching*

This might be a negative point in our synthesis procedure but the antioxidant activity increase at each synthesis steps.

Antibacterial studies
In this study, the antibacterial activity of the synthesized compounds against gram-positive *(Staphylococcus aureus, Bacillus subtilus, Bacillus cereus)* and Gram-negative bacteria (*Escherichia coli, Salmonella typhimurium*) was evaluated by observing the growth inhibition of these tested strains in contact with different concentrations of our compounds (Table 3).

Table 3. *Minimal Inhibitory Concentration and Minimal Bactericidal Concentration of compounds* ***(2-7)***

Products / ***Strains***	***Product* (2)**	***Product* (3)**	***Product* (4)**	***Product* (5)**	***Product* (6)**	***Product* (7)**
S. aureus	0.15 mg·mL^{-1}	-	0.15 mg·mL^{-1}	0.31 mg·mL^{-1}	0.62 mg·mL^{-1}	1.25 mg·mL^{-1}
B. subtilis	0.15 mg·mL^{-1}	-	0.15 mg·mL^{-1}	0.31 mg·mL^{-1}	0.31 mg·mL^{-1}	1.25 mg·mL^{-1}
B. cereus	0.15 mg·mL^{-1}	-	0.07 mg·mL^{-1}	0.15 mg·mL^{-1}	0.15 mg·mL^{-1}	0.62 mg·mL^{-1}
E. coli	0.62 mg·mL^{-1}	-	1.25 mg·mL^{-1}	1.25 mg·mL^{-1}	0.62 mg·mL^{-1}	1.25 mg·mL^{-1}
S. typhi	1.25 mg·mL^{-1}	-	2.55 mg·mL^{-1}	2.55 mg·mL^{-1}	1.25 mg·mL^{-1}	1.25 mg·mL^{-1}

The antibacterial activity tested *in vitro* by microdilution method showed that the tested compounds exhibited an antimicrobial effect against all tested strains except compound

(3) which was devoid of activity (Table 3). It should also be noted that this inhibitory effect depends on the target strain studied. *Bacillus cereus* showed great sensitivity to compound **(4)** and was inhibited from a very low concentration of 0.075 $mg \cdot mL^{-1}$ (Table 3). Also, the concentration of 0.155 $mg \cdot mL^{-1}$ was sufficient to stop the growth of *Bacillus subtilis* and *Staphylococcus aureus*. However, *Escherichia coli* and *Salmonella typhi* have resisted until respective concentrations of **(4)** of 1.25 $mg \cdot mL^{-1}$ and 2.55 $mg \cdot mL^{-1}$. $Gram^{+}$ bacteria proved to be more sensitive to the action of **(4)** than the Gram-negative bacteria which were much more resistant with minimum inhibitory concentrations exceeding 0.625 $mg \cdot mL^{-1}$. Compounds **(2, 4, 5** and **6)** were very effective against Gram positive bacteria and more specifically on *Bacillus cereus*. On the other hand, compounds **(2)** and **(6)** exerted a powerful antibacterial effect on *E. coli* with a MIC of 0.625 $mg \cdot mL^{-1}$.The intrinsic resistance of Gram-negative bacteria is due to the structure of their cell wall which is characterized by the presence of lipopolysaccharides (75 %) having a hydrophilic character which makes the outer membrane of these Gram-negative bacteria impervious to most hydrophobic molecules [36].

Indeed, the results of CMI and CMB are close or equal this means that the compounds almost immediately kill strains = bactericidal.

Several comments can be made for discussing the 2-oxo-1,2-dihydroquinoline-4-carboxylic acid **(2-7)** derivatives results: 1) In this series, the introduction of the ethyl ester group in product **(3)** causes a total loss of activity respectively to all the strains studied. 2) The presence of the N-propargyl and ethoxycarbonyl groups [37], which are clearly very useful (Koga et al. 1980), were best with activity against most of the microorganisms and makes product **(4)** the most active in the series of derivatives studied. 3) The formation of the acid salt (**5)** leads to a decrease in activity respecting to the starting material **(2)**. The di-substitution with propargyl groups **(7)** generally gave decreases in antibacterial potency compared with compounds **(2)** and **(4)** [38, 39].

CONCLUSIONS

In this paper, we have synthesized a new 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives and characterized the compounds by spectral NMR, IR and single crystal X-ray diffraction. After, the antioxidant and antibacterial activities of these compounds were evaluated, and some results are found to have moderate to good activity.

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