

SYNTHESIS OF OXOQUINOLINE DERIVATIVES COUPLED TO DIFFERENT AMINO ACID ESTERS AND STUDYING THEIR BIOLOGICAL ACTIVITY AS CYTOTOXIC AGENTS

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ABSTRACT

Objectives: Quinolines are an important group of organic compounds where several compounds containing a quinoline residue are known to possess useful biological activity and used as antibacterial, antifungal and antitumor agents. These pharmacological properties of quinolines aroused our interest in synthesizing several new compounds featuring heterocyclic rings of the quinoline derivatives linked to amino acid ester side chains with the aim of obtaining a pharmacologically active compounds

Methods: Quinoline was N-alkylated by the bromoacetic acid and then oxidized with an alkaline potassium ferricyanide solution to get N-alkylated quinolone. Conventional solution method for peptide synthesis used as a coupling method between the carboxy-protected amino acids with the acetic acid side chain of quinolone. The DCC/ HOBt coupling reagents used for the peptide bond formation.

Results: The proposed analogues were successfully synthesized and their structural formulas were consistent with the proposed structures as they were characterized and proved by thin layer chromatography (TLC), melting point, infrared spectroscopy (IR) and elemental microanalysis.

All tested analogues showed cytotoxic activity on the HEp-2 cell line (tumor of larynx) with inhibitory concentration percent of (IC %) range (49.01 % - 77.67%).

Conclusion: It can be concluded from the results that the synthesized compounds are promising as new anticancer agents in future.

Keywords: Quinolones, Quinoline anticancer, Quinolines biological activity

INTRODUCTION

Quinoline is one of the most popular N-heteroaromatic compounds incorporated into the structures of many pharmaceuticals. Many quinoline-containing compounds exhibit a wide spectrum of pharmacological activities, such as antiplasmodial [1], cytotoxic [2], antibacterial [3], antiproliferative [4], antimalarial [5], and anticancer activity [6].

These pharmacological properties of quinolines and their derivatives had attracted worldwide attention in the last few decades because of their wide occurrence in natural products and drugs [7,8]. Quinoline derivatives also have been shown to exhibit a wide variety of pharmacological activities including effects on cancer and nowadays it is reported that the incorporation of quinoline nucleus could alter the course of reaction as well as the biological properties of the synthesized compounds [9, 10].

In recent years, large numbers of quinoline derivatives have been synthesized and their various significant biological activities, including different types of cancers, have been reported. The following section provides some examples of novel quinoline derivatives and their cytotoxic properties.

MATERIALS METHODS

Materials

Quinoline was purchased from AVONCHEM (U.K). Absolute ethanol, Absolute methanol, Acetone, and Chloroform were purchased from GCC (Germany). Absolute isopropyl alcohol, Acetic acid, Bromoacetic acid, Diethyl ether, Ethyl acetate, Hydrazine hydrate, Hydrochloric acid, N-methyl morpholine (NMM), Petroleum spirit, Potassium Ferricyanide, Sulfuric acid, and Thionyl chloride were purchased from BDH (U.K). Coumarin was purchased from Himedia (India). D-Alanine, Glycine, 1-Hydroxy benzotriazole (HOBt), L-cysteine, L-Histidine methyl ester 2HCl, L-Leucine, L-Phenylalanine, L-Tyrosine, and N,N-Dimethyl formamide (DMF), were purchased from Fluka AG (Switzerland). All other reagents were of analytical grade

Methods of Identification

General methods were used for purification and identification of the synthesized analogues including:

- Thin Layer Chromatography:

Ascending thin layer chromatography was run for monitoring the reaction progress as well as checking the purity of our products. The compounds were revealed by reactivity with iodine vapor.

- Melting Points:

Thomas Hoover Electronic Melting Point Apparatus was used to determine all melting points reported in this work.

- Infrared Spectra:

Determinations of infrared spectra were recorded by KBr film FTIR shimadzu (Japan).

- Elemental Microanalysis:

Elemental microanalysis was done by using Carloarba elemental analyzer in Cleveland Clinical Foundation Learner Research Institute - France.

Synthesis

Esterification of Amino Acids

Synthesis of L-Tyrosine ethyl ester HCl (Tyr-O-Eth) Compound (A1)

A suspension of Tyrosine (50 mmol, 9.05g) in (150 ml) of absolute ethanol, was cooled down to -15°C then thionyl chloride (50 mmol, 3.7 ml, 5.95 g) was added drop wise, (the temperature should be kept below -10°C), and the reaction mixture was left at 40°C for 3hr, then reflux started for 3hr and left at (R. T.) overnight. The solvent was evaporated to dryness under vacuum, redissolved in methanol and evaporated; this process was repeated several times and recrystallizes from methanol-ethyl acetate (3:1) [11].

The following compounds were synthesized using the same method of compound A1:

- Glycine ethyl ester HCl(Gly-o-Et) compound (A2)
- L-phenyl alanin ethyl ester HCl(Phe-O-Et) compound (A3)
- D-Alanine methyl ester HCl(D-Ala-O-Me) compound (A4)

Synthesis of quinolinium bromide N acetic acid

Bromoacetic acid (0.2 mole, 27.79 g.) dissolved in abs. ethanol then quinoline (0.2 mole, 25.83 g.) added and reflux started at 70 °C for 6 hr. and left at R.T. for 24 hr, the product appear as ppt, after decant of the supernatant liquid, the ppt. washed with ethanol three times then filter and finally recrystallized from ethyl acetate- chloroform mixture (1:1).

Synthesis of quinoline derivatives

• Synthesis of 2-quinolone N acetic acid (D)

Oxidation of quinolinium bromide N acetic acid, 0.07 mole (18.76 g.) dissolved in water (80 ml.)

Solution of sodium hydroxide 80ml (0.42 mole, dissolved in water), and potassium ferricyanide ,200ml.(0.14 mole, dissolved in hot water) were prepared.

At a day interval 20ml. portion of sodium hydroxide and 25 ml of potassium ferricyanide were added to the quinolinium bromide salt solution with stirring after each addition. The heat of mixture kept the reaction slightly above room temperature.

After addition of the final 25 ml. portion of potassium ferricyanide, the reaction mixture was kept warm for one hr. and left overnight then acidified with 6N HCl. Granular crystals ppt and were removed by filtration recrystallization from hot ethanol [12].

Synthesis of 2-quinolone N acetic acid Ethyl ester HCl (D1)

A suspension of 2-quinolone N acetic acid (0.25mmol, 50.8mg) in (20 ml) of absolute ethanol, was cooled down to -15°C then complete the procedure as mentioned in the synthesis of A1.

Synthesis of 2-quinolone N acetic acid Isopropyl ester HCl (D2)

A suspension of 2-quinolone N acetic acid (0.25mmol, 50.8mg) in (20 ml) of absolute isopropyl alcohol, was cooled down to -15°C then thionyl chloride (0.25mmol, 0.02ml.) was added drop wise, Then complete the procedure as mentioned in the synthesis of A1.

Coupling Method & Reagents

Conventional solution method for peptide synthesis was used as a coupling method between the carboxy protected amino acids and carboxy derivatives of quinolone. DCC was used in the peptide bond formation as the coupling reagent, while HOBT was used to decrease racemization and to increase the yields [13].

Synthesis of 2-quinolone N acetyl -L-Tyr ethyl ester (D3)

To a stirred solution of Tyr ethyl ester HCl (compound A1) (0.3mmol, 74mg) in (30ml) of DMF, (0.3mmol, 0.04ml) of NMM was added with stirring for 10 min., then (0.3mmol, 61mg) of (comp D) was also added, and the mixture was cooled down to (-10°C) then (0.6mmol, 81mg) of HOBT and (0.3mmol, 62mg) of DCC were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5 days.

The reaction mixture evaporated to exclude DMF and redissolved in chloroform from which the N,N-Dicyclohexyl urea (DCU) was filtered off. The clear filtrate washed twice with 5% sodium bicarbonate solution, 0.1N HCl, once with water, and with saturated sodium chloride solution. The chloroform layer was dried with anhydrous magnesium sulphate and evaporated under vacuum; the resulted product was collected, recrystallized from (methanol: chloroform) (5:1) [14].

Synthesis of 2-quinolone N acetyl -Gly ethyl ester (D4)

To a stirred solution of Gly ethyl ester HCl (compound A2) (0.3mmol, 41.9mg) in (30ml) of DMF, (0.3mmol, 0.04ml) of NMM was added with stirring for 10 min., then (0.3mmol, 61mg) of (comp D) was also added, and the mixture was cooled down to (-10°C) then (0.6mmol, 81mg) of HOBT and (0.3mmol, 62mg) of DCC were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5days. Then complete the procedure as mentioned in the synthesis of D3 [14].

Synthesis of 2-quinolone N acetyl -Phe ethyl ester (D5)

To a stirred solution of Phe ethyl ester HCl (compound A3) (0.3mmol, 68.9mg) in (30ml) of DMF, (0.3mmol, 0.04ml) of NMM was added with stirring for 10 min., then (0.3mmol, 61mg) of (comp D) was also added, and the mixture was cooled down to (-10°C) then (0.6mmol, 81mg) of HOBT and (0.3mmol, 62mg) of DCC were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5days. Then complete the procedure as mentioned in the synthesis of D3 [14].

Synthesis of 2-quinolone N acetyl -D-Ala methyl ester (D6)

To a stirred solution of D-Ala methyl ester HCl (compound A4) (0.3mmol, 41.9mg) in (30ml) of DMF, (0.3mmol, 0.04ml) of NMM was added with stirring for 10 min., then (0.3mmol, 61mg) of (comp D) was also added, and the mixture was cooled down to (-10°C) then (0.6mmol, 81mg) of HOBT and (0.3mmol, 62mg) of DCC were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5days. Then complete the procedure as mentioned in the synthesis of D3 [14].

Cytotoxic Assay

The *in vitro* cytotoxicity assays with cultured cells are widely used to evaluate chemicals including cancer chemotherapeutics, pharmaceuticals, biomaterials, natural toxins, antimicrobial agents and industrial chemicals because they are rapid and economical [15].

The cytotoxic effect of our analogues was evaluated by MTT assay; a non-radioactive, fast assay widely used to quantify cell viability and proliferation. MTT is a yellow water soluble tetrazolium salt, metabolic active cells are able to convert the dye to water insoluble dark blue formazan by reductive cleavage of tetrazolium ring that can be detected through UV light to give us a correlation between optical density and viable cells counts. A set of two fold in three concentrations (125, 250, 500 µg/ml) was made for each product and the exposure time of the assay was 72hrs.

Effect of Samples on HEp-2 Cell Line

Human Epidermoid Larynx Carcinoma (HEp-2) Cell Line was used in the Study. The cell line was kindly provided by Iraqi Center for Cancer and Medical Genetic Research. This human cell line was originally come from a 57-year-old man with a primary tumor of the larynx. It was implanted in irradiated and cortisone treated rat. After growth in the rat, the tumor was excised and implanted as an *in vitro* tissue culture. HEp-2 cells grew rapidly, doubling themselves in 2-3 days. Passages (235) were used throughout this study.

RESULT AND DISCUSSION

Synthetic Part

A. The reaction pathways:

The aim of our research is to synthesize quinoline derivatives coupled to different amino acid esters (Scheme 1).

The overall synthesis strategy based one four major lines:

1. Amino acid derivatives

The amino acids were activated by thionyl chloride to get acyl chloride that attacks either ethanol or methanol to get ethyl or methyl esters of the selected amino acids

2. Oxoquinoline derivatives

Quinoline was N-alkylated by the α - halo carboxylic acid and then oxidized through the alkaline potassium ferricyanide solution to get N-alkylated quinolone.

3. Peptide bond formation

Conventional solution method for peptide synthesis used as a coupling method between the carboxy-protected amino acids or 1-amino quinolone derivatives with acetic acid side chain of quinolone. The DCC/ HOBT coupling reagents used for peptide bond formation.

The overall reaction pathway is shown in the following scheme (figure 1):

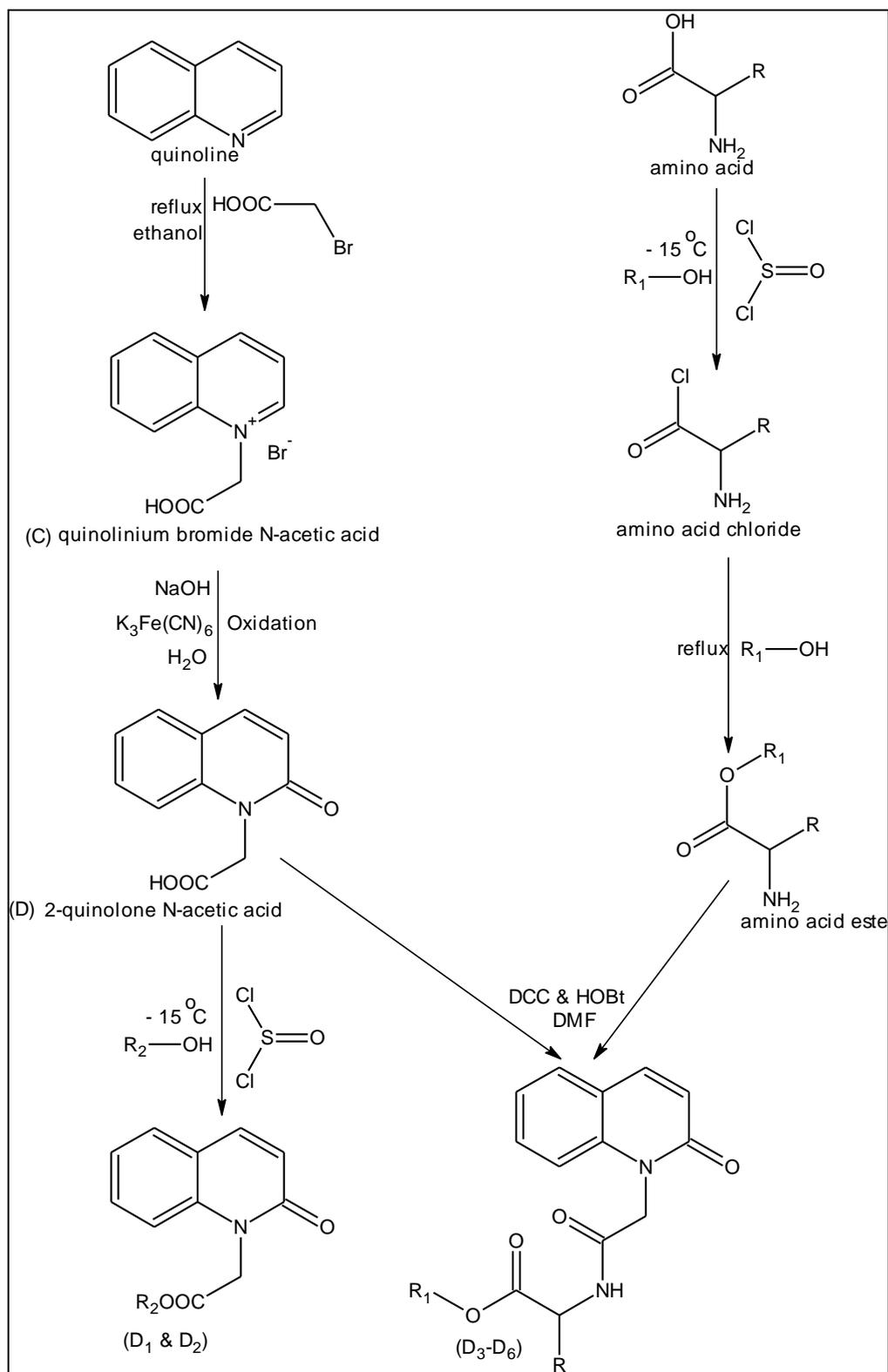


Fig.1: Scheme of overall pathway of synthesis of quinoline derivatives where R= -H, -CH₃, -CH₂-C₆H₅, -CH₂-C₆H₄-OH, R₁= Me or Et, R₂= Et or Isopropyl

B. Strategy of synthesis

The strategy of synthesis started from the esterification of amino acids then proceeded to the synthesis of 2-quinolone N acetic acid from quinoline and finally coupling between the 2-quinolone N acetic acid and the amino group of the amino acid esters, and as follows:

1) Esterification of amino acids

The esterification of carboxyl group of amino acids is normally used as an amino acid protecting group. Esterification of carboxyl group enhances the nucleophilic character of amine group and allows its subsequent acylation [16].

2) Alkaline potassium ferricyanide oxidation

It is generally accepted that the oxidation takes place *via* the pseudo base. The mechanism of the reaction probably proceeds in two steps:

1st step: Formation of the pseudo-base by an aq. NaOH

When quinoline is heated (225-300 °C) with KOH or NaOH-KOH, 2-quinolone is produced together with a nearly-quantitative yield of hydrogen. By far the most important aspect of the reactivity of salts of quinoline is the greatly enhanced the susceptibility to addition of a nucleophile to C2. The simple alkyl quinolinium salts undergo addition reaction easily, even with weak nucleophile as hydroxide ion, therefore the NaOH hydroxylation occur at room temperature [17] as shown in (figure 2).

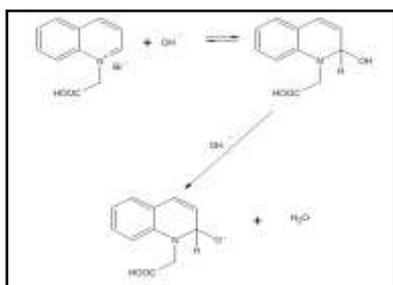


Fig. 2: Scheme of the hydroxylation of quinolinium salt

2nd step: The dehydrogenation of the pseudo-base to form quinolone derivative as shown in (figure 3) [17].

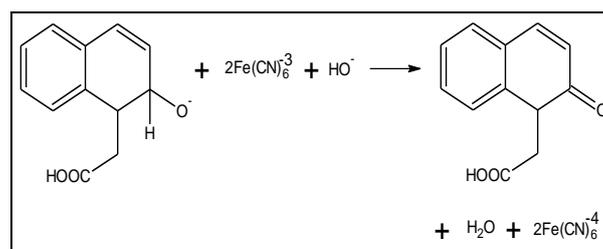


Fig. 3: Scheme of the potassium ferricyanide oxidation

3) Peptide Coupling Method:

There is many ways to form a peptide bond [18], however in this work; the direct coupling with DCC/HOBt method was used.

This method is characterized as being simple, efficient, no racemization, rapid, and leading to a good yield at R. T. [19]. The overall reaction resembles the dehydration process to form the amide bond.

The properties of synthesized new compounds are shown in table (1) while the characteristic IR bands are shown in table (2) and figure (4&5). On the other hand the data obtained from elemental microanalysis are shown in table (3).

Table 1: The identification parameters of the synthesized compounds

No	compound code	Name	Solvent system	Yield %	Physical appearance	m.p °C	Rf
1	C	Quinolinium Bromide N-Acetic acid	CH ₃ OH 7 H ₂ O 3	66.6	yellow crystals	199	0.44
2	D	2-Quinolone N-Acetic acid	CH ₃ OH 8 H ₂ O 2	56	Brown crystals	220	0.87
3	D1	2-Quinolone N-Acetic acid ethyl ester	CH ₃ OH 8 H ₂ O 2	96	Brown crystals	144	0.92
4	D2	2-Quinolone N-Acetic acid isopropyl ester	CH ₃ OH 8 H ₂ O 2	95	Brown crystals	141	0.93
5	D3	2-Quinolone N-Acetyl -Tyr-O-Eth	CH ₃ OH 5 CHCl ₃ 5	95	Brown crystals	151	0.93
6	D4	2-Quinolone N-Acetyl -Gly-O-Eth	CH ₃ OH 8 CHCl ₃ 2	91	Brown crystals	139	0.89
7	D5	2-Quinolone N-Acetyl -Phe -O-Eth	CH ₃ OH 8 CHCl ₃ 2	93	Brown crystals	118	0.95
8	D6	2-Quinolone N-Acetyl -D-Ala-O-Me	CH ₃ OH 8 CHCl ₃ 2	89	Brown crystals	168	0.92

Table 2: The characteristic IR bands of the synthesized compounds

No	compound	Name	Characteristic IR bands
1	C	Quinolinium Bromide N-Acetic acid	(3200-2410 OH), (2978 assy. CH ₂), (2831 symm.CH ₂) (1736 C=O of COOH), (1365 CH ₂ bend), (1195 C-O)
2	D	2-Quinolone N-Acetic acid	3300-2410 OH), (2970 assy. CH ₂), (1728 C=O of COOH), (1666 C=O of 2-quinolone) (1453 CH ₂ bend) (1350 CH ₂ bend), (1211 C-O)
3	D1	2-Quinolone N-Acetic acid ethyl ester	(2978 assy. CH ₃), (2939 assy.CH ₂) (1744 C=O of Ester), (1666 C=O of 2-quinolone) (1458 CH ₂ bend) (1365 CH ₂ bend), (1203 C-O)
4	D2	2-Quinolone N-Acetic acid isopropyl ester	(2972 assy. CH ₃), (2933 assy.CH ₂) (1736 C=O of Ester), (1682 C=O of 2-quinolone) (1465 CH ₂ bend) (1380 1370 Gem dimethylCH ₃ bend), (1211 C-O)
5	D3	2-Quinolone N-Acetyl -Tyr-O-Eth	(3326 NH, OH), (2932 assy. CH ₂), (2854 symm.CH ₃) (1740 C=O of Ester), (1666 C=O peptide, amide I) (1628 C=O of 2-quinolone), (1574 NH bend, amide II) (1450 CH ₂ bend),
6	D4	2-Quinolone N-Acetyl -Gly-O-Eth	(3326 NH), (2932 assy. CH ₂), (2854 symm.CH ₃) (1745 C=O of Ester), (1674 C=O peptide, amide I) (1628 C=O of 2-quinolone), (1574 NH bend, amide II) (1450 CH ₂ bend)
7	D5	2-Quinolone N-Acetyl -Phe -O-Eth	(3325 NH), (2932 assy. CH ₂), (2854 symm.CH ₃) (1741 C=O of Ester), (1665 C=O peptide, amide I) (1628 C=O of 2-quinolone), (1574 NH bend, amide II) (1450 CH ₂ bend)
8	D6	2-Quinolone N-Acetyl -D-Ala-O-Me	(3325 NH), (2932 assy. CH ₂), (2854 symm.CH ₃) (1745 C=O of Ester), (1674 C=O peptide, amide I), (1628 C=O of 2-quinolone) (1450 CH ₂ bend), (1574 NH bend, amide II)

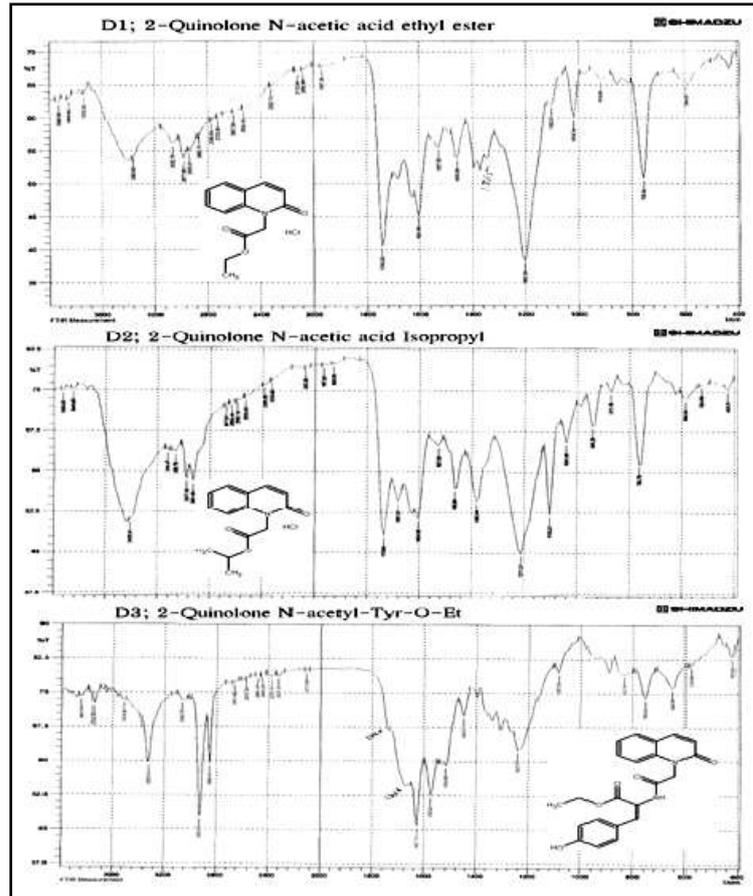


Fig. 4: IR spectra of synthesized compounds D1, D2, and D3

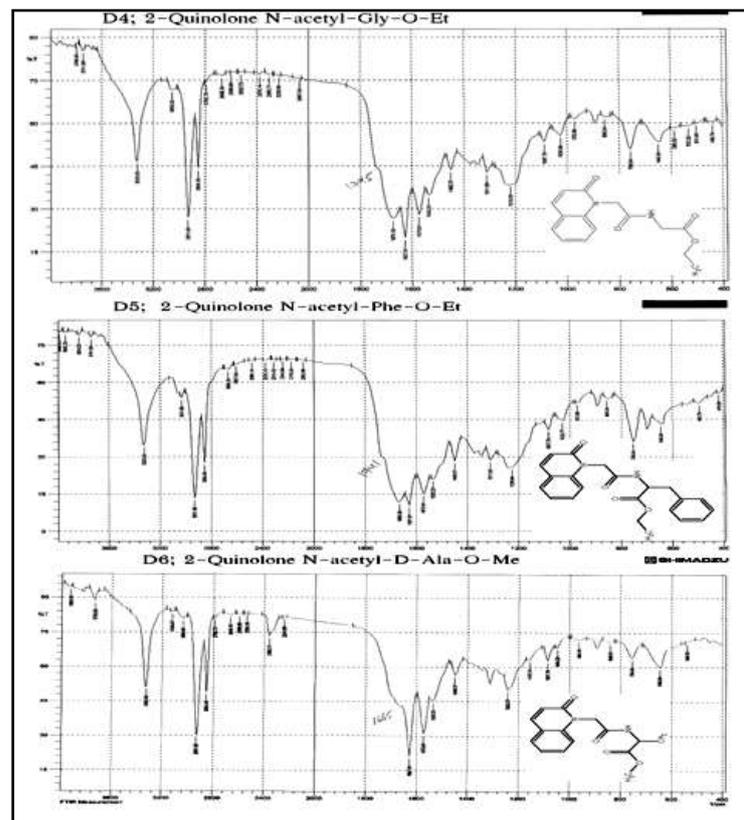


Fig. 5: IR spectra of synthesized compounds D4, D5, and D6

Table 3: The elemental microanalysis of the synthesized compounds

Compound symbol	Value type	C	H	N	O	Cl	Molecular weight
D	calculated	65.025	4.433	6.897	23.645		203
	observed	65.025	4.433	6.897	23.645		
D1	calculated	58.318	5.234	5.234	17.944	13.271	267.5
	observed	58.576	5.333	5.401	18.207	13.483	
D2	calculated	59.680	5.684	4.973	17.052	12.611	281.5
	observed	59.981	5.731	5.303	17.510	12.999	
D3	calculated	67.005	5.584	7.107	20.304		394
	observed	67.055	5.698	7.391	20.578		
D4	calculated	62.500	5.556	9.722	22.222		288
	observed	62.849	5.633	9.921	22.531		
D5	calculated	69.841	5.820	7.408	16.931		378
	observed	70.093	6.009	7.701	17.245		
D6	calculated	62.500	5.556	9.722	22.222		288
	observed	62.801	5.711	9.961	22.998		

Table 4: Cytotoxic effect of the samples by comparing their optical density with the control

Compound	125µg O.D.	250µg O.D.	500 µg O.D.	Control O.D.	IC% 125µg	IC% 250µg	IC% 500 µg
D	0.108	0.107	0.131	0.271	60.15%	60.52%	51.66%
D1	0.119	0.111	0.113	0.271	56.01%	59.0%	58.5%
D2	0.108	0.110	0.115	0.271	60.15%	59.41%	57.56%
D3	0.119	0.118	0.126	0.271	56.01%	56.46%	53.5%
D4	0.137	0.120	0.138	0.271	49.45%	55.72%	49.01%
D5	0.115	0.122	0.151	0.515	77.67%	76.31%	70.68%
D6	0.104	0.107	0.137	0.271	61.62%	60.52%	49.45%

Summary of cytotoxic study Results

The cytotoxic study was done on Hep-2 cell line passage (235)

Exposure time =72hrs.

Staining is MTT stain

Replication number is 3

Control number 2

IC% Inhibitory concentration = $[(\text{Control O.D.} - \text{Sample O.D.}) / (\text{Control O.D.})] \times 100$

When the cancer cell line (HEp-2) was treated with these products the result showed significant cytotoxic effect in all tested samples in comparison with the control. The toxic effect varied from one sample to another, all samples showed a significant toxicity ($P < 0.05$) started from 125µg/ml to the 500µg/ml.

The inhibitory concentration percent (IC %) was estimated, and the result was varied among samples as shown in table (4).

CONCLUSIONS

Six new quinoline derivatives with high purity and promising cytotoxic effect were synthesized and can be consider as a good anticancer drug candidate

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