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 quinoline. Conventional solution method for peptide synthesis used as a coupling method between the carboxy-protected amino acids with the acetic acid side chain of quinoline. 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 antitumor 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, Broumacetic acid, Diethyl ether, Ethyl acetate, Hydrazine hydrate, Hydrochloric acid, N-methyl morpholine (NMM), Petroleum spirit, Potassium Ferricyanide, Sulphuric acid, and Thiouyl chloride were purchased from BDH (U.K). Coumarin was purchased from Himedia (India). D-Alanine, Glycine, 1-Hydroxy benzotriazole (HOBr), L-cysteine, L-Histidiine 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 Carlsraha 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 thiouyl 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, dissoved in methanol and evaporated; this process wa...
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 quinoline. 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, 7.4mg) in (30ml) of DMF, (0.3mmol, 0.04ml) of NMM was added with stirring for 10 min, then (0.3mmol, 6.1mg) 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, 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-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, 91mg) 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 on 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 quinoline.
3. Peptide bond formation
Conventional solution method for peptide synthesis used as a coupling method between the carboxy-protected amino acids or 1-amino quinoline derivatives with acetic acid side chain of quinoline. 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 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

Table 1: The identification parameters of the synthesized compounds

<table>
<thead>
<tr>
<th>No</th>
<th>compound code</th>
<th>Name</th>
<th>Solvent system</th>
<th>Yield %</th>
<th>Physical appearance</th>
<th>m.p. °C</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Quinolinium Bromide N-Acetic acid</td>
<td>CH3OH : H2O 4</td>
<td>66.6</td>
<td>yellow crystals</td>
<td>199</td>
<td>0.44</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>2-Quinolone N-Acetic acid</td>
<td>CH3OH : H2O 4</td>
<td>56</td>
<td>Brown crystals</td>
<td>220</td>
<td>0.87</td>
</tr>
<tr>
<td>3</td>
<td>D1</td>
<td>2-Quinolone N-Acetic acid ethyl ester</td>
<td>CH3OH : H2O 4</td>
<td>96</td>
<td>Brown crystals</td>
<td>144</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
<td>D2</td>
<td>2-Quinolone N-Acetic acid isopropyl ester</td>
<td>CH3OH : H2O 4</td>
<td>95</td>
<td>Brown crystals</td>
<td>141</td>
<td>0.93</td>
</tr>
<tr>
<td>5</td>
<td>D3</td>
<td>2-Quinolone N-Acetyl-Tyr-O-Eth</td>
<td>CH3OH : H2O 4</td>
<td>95</td>
<td>Brown crystals</td>
<td>151</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>D4</td>
<td>2-Quinolone N-Acetyl -Gly-O-Eth</td>
<td>CH3OH : H2O 4</td>
<td>91</td>
<td>Brown crystals</td>
<td>139</td>
<td>0.89</td>
</tr>
<tr>
<td>7</td>
<td>D5</td>
<td>2-Quinolone N-Acetyl –Phe -O-Eth</td>
<td>CH3OH : H2O 4</td>
<td>93</td>
<td>Brown crystals</td>
<td>118</td>
<td>0.95</td>
</tr>
<tr>
<td>8</td>
<td>D6</td>
<td>2-Quinolone N-Acetyl –D-Ala-O-Me</td>
<td>CH3OH : H2O 4</td>
<td>89</td>
<td>Brown crystals</td>
<td>168</td>
<td>0.92</td>
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</table>

Table 2: The characteristic IR bands of the synthesized compounds

<table>
<thead>
<tr>
<th>No</th>
<th>compound code</th>
<th>Name</th>
<th>Characteristic IR bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Quinolinium Bromide N-Acetic acid</td>
<td>(3200-2410 O-H), (2978 assy. CH2), (2831 symm.CH2), (1736 C=O of COOH), (1365 CH2 bend), (1195 C-O)</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>2-Quinolone N-Acetic acid</td>
<td>3300-2410 O-H), (2970 assy. CH2), (1728 C=O of COOH), (1666 C=O of 2-quinolone)</td>
</tr>
<tr>
<td>3</td>
<td>D1</td>
<td>2-Quinolone N-Acetic acid ethyl ester</td>
<td>(1453 CH2 bend) (1350 CH2 bend), (1211 C-O)</td>
</tr>
<tr>
<td>4</td>
<td>D2</td>
<td>2-Quinolone N-Acetic acid isopropyl ester</td>
<td>(2978 assy. CH3), (2939 assy.CH2)</td>
</tr>
<tr>
<td>5</td>
<td>D3</td>
<td>2-Quinolone N-Acetyl-Tyr-O-Eth</td>
<td>(1734 C=O of Ester), (1666 C=O of 2-quinolone), (1458 CH2 bend)</td>
</tr>
<tr>
<td>6</td>
<td>D4</td>
<td>2-Quinolone N-Acetyl -Gly-O-Eth</td>
<td>(1682 C=O of Ester), (1465 CH2 bend)</td>
</tr>
<tr>
<td>7</td>
<td>D5</td>
<td>2-Quinolone N-Acetyl –Phe -O-Eth</td>
<td>(1211 C-O)</td>
</tr>
<tr>
<td>8</td>
<td>D6</td>
<td>2-Quinolone N-Acetyl –D-Ala-O-Me</td>
<td>(1195 C-O)</td>
</tr>
</tbody>
</table>
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**

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

**Summary of cytotoxic study Results**

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

Exposure time = 72 hrs.

Staining is MTT stain

Replication number is 3

Control number 2

IC% Inhibitory concentration = [(Control O.D. – Sample O.D.) / (Control O.D.)] x 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 considered as a good anticancer drug candidate.

**REFERENCES**


