Alkaloids 8-Hydroxyquinoline derivatives: Synthesis and biological activities

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Abstract
In this work, six new alkaloids have been synthesized from 8-hydroxyquinoline, the other part is to realize the biological activities of these compounds such as antibacterial activity, antifungal activity and cytotoxicity. The antibacterial activity was carried out by the method of diffusion in agar against: Staphylococcus aureus, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The antifungal activity was carried out by the method of diffusion in agar against: Botrytis cinerea, Fusarium oxysporum and Verticillium albo atrum, and cytotoxicity activity was achieved a larvae Artemia salina (Brine shrimp). The overall results show that the new alkaloids compounds exhibit remarkable biological activity.

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1. Introduction:
Alkaloids are part of the most important compounds among heterocyclic compounds found their broad application in pharmaceutical, cosmetic and agrochemical industries [1-3]. These compounds represent one of the most vast classes of natural products, and they have attracted considerable attention due to their biological activities [4-7]. Alkaloids are also important synthetic intermediates in preparing a variety of biologically active compounds [8-10].

In the other hand, Interest in 8-Hydroxyquinoline ¹ is indicated by the great number of reports dealing with their numerous derivatives synthesis [11-13]. The biological activity of these derivatives depends not only on the bicyclic hetero-aromatic pharmacophore but also on the nature of the peripheral substituent and their spatial relationship. They have been reported to possess biological activities: antibacterial [14], antifungal [15], antiviral [16], anticancer [17], antidyshlipidemic [18], antioxidative [19], anti-inflammatory [20], and Antileishmanial activities [21].

We report in this paper, the synthesis and the biological activities (antibacterial activity, antifungal activity and cytotoxicity) of series alkaloids 8-hydroxyquinoline ¹ derivatives: 2.a, 2.b, 2.c, 3, 4 and 5 (Figure1).

2. Material and methods:
2.1. General:
All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined with a digital melting point apparatus SMP 10. NMR spectra were taken on a Bruker Avance II + 300 MHz spectrometer, operating at 300 MHz for ¹H, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements were carried out at ambient temperature. The FTIR spectra were recorded with a VERTEX 70 FT-IR spectrometer (Bruker Optics). The crystals were stirred with KBr. The spectra are from 4000 cm⁻¹ to 400 cm⁻¹ at resolution 2 cm⁻¹ with 49 scans. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F₂₅₄, 0.2 mm Merck plates, eluent system Acetonitril:Ethanol (gradient).

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2.2. Synthesis of compounds:

2.2.1. Synthesis of compounds 2a, 2b and 2c:

2a was prepared by mixture 14.6 g (63.5 mmol) of compound 1, with 15 ml of concentrated hydrochloric acid and 16 ml (577.6 mmol) of 37% formaldehyde for 90 minutes at degassing HCl gaz. next steep, 2b was prepared by mixing 0.7g (3.04 mmol) of 2a and 25 ml of a solution of 0.84g NaOH (21 mmol). Finally, 7.72 g (20 mmol) cholesterol was added to 4.14 g (30 mmol) of potassium carbonate and 50 ml of anhydrous DMSO for 4 hours, immediately the resulting mixture added to a solution of compound 2a in DMSO (4.6 g, 20 mmol), and it is maintained in normal conditions with stirring overnight. After neutralization of the mixture with a solution of HCl, the product 2c was crystallized.

**Compound 2a:** yield: 78%; color: yellow; m.p. = 285 °C; IR (KBr) cm⁻¹: 3420, 2863, 2010, 1900, 1640, 1600, 1560, 1500, 1400, 1360; ¹H NMR (300 MHz, DMSO-d₆): δ 9.34 (d,1H, CH), 9.04 (d,1H, CH), 8.08 (d,1H, CH), 7.72 (d,1H, CH), 7.34 (d,1H), 5.35 (s,1H, OH), 5.22 (s,2H, CH₂).

**Compound 2b:** yield: 73%; color: green; m.p. > 285 °C; IR (KBr) cm⁻¹: 3450, 3200, 2859, 1635, 1600, 1583, 1510. ¹H NMR (300 MHz, DMSO-d₆): δ 8.75 (d,1H, CH), 8.12 (d,1H, CH), 7.76 (m,1H, CH), 7.16 (d,1H, CH), 7.14 (d,1H, CH), 5.46 (s,1H, OH), 4.61 (d,2H, CH₂), 3.76 (s,1H, OH).

**Compound 2c:** yield: 55%; color: green; m.p. = 90 °C; IR (KBr) cm⁻¹: 3420, 2160, 2880, 1500, 1460, 1380, 1350. ¹H NMR (300 MHz, DMSO-d₆): δ 9.10 (d,1H, CH), 8.42 (d,1H, CH), 8.15 (t,1H, CH), 7.23 (d,1H, CH), 6.78 (d,1H, CH), 5.39 (s,1H, OH), 4.67 (s,1H, CH₂), 3.56 (m,1H), 0.7-2.5 (m).

2.2.2. Synthesis of compound 3:

A solution of 15g (103 mmol) of compound 1, with 60 ml of ethanol, and 28 mL of concentrated sodium hydroxide solution was heated at 100 °C with agitation for 12 h and cooled to room temperature. After this steep was added 14g (117 mmol) chloroform (the reaction is exothermic). Then the mixture was evaporated, acidified with HCl and then extracted with chloroform. Immediately after the evaporation of the organic phase was added a mixture chloroform / ethanol and this solvent was evaporated and filtrated, the residue (compound 3) was purified by crystallization.

**Compound 3:** yield: 31%; color: brown; m.p. = 250 °C; IR (KBr) cm⁻¹: 3460, 1620, 1520; ¹H NMR (300 MHz, DMSO-d₆): δ 8.62 (s,1H, CH), 8.56 (d,1H, CH), 8.18 (d,1H, CH), 7.76 (m,1H, CH), 7.14 (d,1H, CH), 6.66 (d,1H, CH), 5.28 (s,1H, OH).

2.2.3. Synthesis of compound 4:

Step 1: A mixture 5.68g (20 mmol) of stearic acid with 4.4ml (60 mmol) of thionyl chloride was heated at 45 °C -50 °C for 30 min, then the mixture was mounted 90 - 95 °C in 15 min. The resulting mixture is degassed under vacuum to remove HCl and SO₃, then distillation was done to purify the acid chloride.

Step 2: A mixture of 2.9 g (20 mmol) of compound 1 was dissolved in anhydrous tetrahydrofuran and an excess of anhydrous aluminum chloride (30 mmol), the mixture was then added to the acid chloride prepared in the first step. At the end of this addition, the mixture was agitated for one hour. After cooling the reaction mixture, the organic phase was washed with NaOH (C = 1N), then it was washed by water. Immediately, a drying phase by anhydrous calcium chloride, and evaporation of the solvent there is obtained the compound 4.

**Compound 4:** yield: 57%; color: brown; m.p. = 52 °C; IR (KBr) cm⁻¹: 3400, 3050, 2940, 2880, 1740, 1620, 1600, 1500, 1480, 1420, 1390, 1360; ¹H NMR (300 MHz, DMSO-d₆): δ 8.72 (d,1H, CH), 8.44 (d,1H, CH), 7.82 (t,1H, CH), 7.16 (d,1H, CH), 6.83 (d,1H, CH), 5.38 (s,1H, OH), 2.35 (t,2H, CH₂), 1.63 (m,2H CH₂), 1.3 (s,28H, CH₂), 0.89 (t,3H, CH₃).

2.2.4. Synthesis of compound 5:

Three solutions: S1, S2 and S3 were prepared at a temperature between 0 and 5 °C. The first solution S1 was prepared by adding 1.08 g (15.6 mmol) of sodium nitrite in 10 ml of cooled water. The second S2 was prepared by adding 1.29 ml (14.2 mmol) of 5-fluoroaniline in 4 ml of concentrated hydrochloric acid. The latter solution was prepared by adding 1.56 g (14.2 mmol) of A in the 10% sodium hydroxide. Finally, was added dropwise S1 to S2, then to add the mixture obtained in S3, after filtration is obtained the compound G and was purified by crystallization.

**Compound 5:** yield: 62%; color: green; m.p. > 285 °C; IR (KBr) cm⁻¹: 3610, 3380, 1590, 1570, 1550, 1480, 1460, 1410, 1380, 1340; ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 6.50 (d,1H, CH), 7.25 (t,2H, CH), 7.45 (m,1H, CH), 7.80 (d,1H, CH), 7.82 (t,2H, CH), 8.06 (d,1H, CH), 9.15 (d,1H, CH).
2.3. Biological activities:
2.3.1. Antibacterial activity:
The method used is the well diffusion agar described by Aianne et al. (2014) [22]. This method can quickly observe effects of a substance by bacterial growth. Screening for antibacterial activity of the compounds was determined by agar well diffusion method. The extracts was dissolved in dimethyl sulfoxide (DMSO) 5%. Ten microliter of crude extract (2 mg/mL) was loaded onto well (diameter 6 mm). Fresh colonies of Streptococcus faecalis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa on supplemented MH agar were inoculated in supplemented MH broth and incubated overnight under aerobic condition. The bacterial suspensions were adjusted to McFarland standard No. 0.5 and spreaded onto supplemented MH agar plates. The seeded plates and incubated at 37 °C for 24 h under aerobic condition. The diameters of the inhibition zones were measured and the mean was recorded. Experiments were done in triplicate. Bacterial culture with 1% DMSO was used as negative control. In addition, Tetracycln and Streptomycin used a positive control.

2.3.2. Antifungal activity:
The method used is the well diffusion agar described by Maadane et al. (2017) [23]. Briefly to determine the inhibitory effects of compounds. The compounds were incorporated into the PDA and poured into petri dishes. Agar discs (0.3 cm in diameter) covered with germinated fungal conidia, served as a source of inoculums. These agar discs were placed in the center of petri dishes containing PDA with the corresponding compounds at concentration 50 µg/disk. Petri dishes were sealed with parafilm to prevent the leak of test compounds. The plates without the compounds were used as control, and Amphotericin B used a positive control. For spore germination assays, spore suspensions of each fungus (10^3 conidia/ml counted with a hemocytometer) were prepared in sterile water. 0.1 ml of each suspension was added to Petri dishes containing PDA with and without the test compounds. The fungi growth was diameter measured after 15 days of incubation at 25°C. Three replicates were used for each compound.
2.3.3. Cytotoxicity test:
The cytotoxicity test Brine Shrimp used in this work is described by Ainane et al. (2018) [24]. Briefly, this test determines the toxic activity of the products tested their effects on the larvae of brine shrimp: Artemia salina. It also allows for determining the median lethal dose LD$_{50}$ which can determine the power of toxicity compared to other products of references. Test samples are dissolved in 2% DMSO, with concentrations of 20, 40, 60 and 100 µg / ml and, immediately determined volumes of the prepared solution are added to tubes containing the larvae of Artemia salina. The tubes are placed in a chamber at room temperature and the results were read after 24 hours is by counting under a dissecting microscope. If the lamp contains dead larvae, the percentage mortality is corrected using the following formula (1):

$$\% \text{M} = \frac{\text{NLP}}{\text{NLT}} \times 100$$

With:
- $\% \text{M}$: Percent mortality.
- NLP: Number of dead larvae in the Presence of the Product.
- NLT: Number of dead larvae in the Presence of Control (solvent).

3. Results and Discussion:
Compound 2.a was prepared according Yamato (1987) [25] from 8-hydroxyquinoline 1 with the reagents: formaldehyde and hydrochloric acid, straight away, the compound 2.b was prepared by hydrolysis of compound 2.a in the presence of sodium hydroxide. On the other hand, the compound 2.c was prepared by grafting cholesterol of compound 2.a. The compounds 3 and 4 were prepared using compound 1 according to the reactions successively Reimer-Tiemann [26] and Friedel-Crafts [27]. Finally the synthesis of compound 5 was obtained by fixing compound 1 on divalent nitrogen of 4-fluoroaniline [28].

The antibacterial activity was carried out by four bacteria: *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. the method used was the disc method on agar diffusion, to determine the diameter of inhibition induced by compounds 1, 2.a, 2.b, 2.c, 3, 4 and 5 compared to each compound being tested at a concentration of 20 µg / disk, in parallel we tested tetracycline and streptomycin two antibiotics (positive control). The results obtained in this work are shown in Table 1.

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>S. faecalis</em></th>
<th><em>E. coli</em></th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35 mm</td>
<td>20 mm</td>
<td>20 mm</td>
<td>5 mm</td>
</tr>
<tr>
<td>2.a</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2.c</td>
<td>6 mm</td>
<td>3 mm</td>
<td>7 mm</td>
<td>3 mm</td>
</tr>
<tr>
<td>3</td>
<td>27 mm</td>
<td>25 mm</td>
<td>20 mm</td>
<td>30 mm</td>
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<tr>
<td>4</td>
<td>3 mm</td>
<td>10 mm</td>
<td>5 mm</td>
<td>4 mm</td>
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<tr>
<td>5</td>
<td>15 mm</td>
<td>13 mm</td>
<td>25 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15 mm</td>
<td>13 mm</td>
<td>20 mm</td>
<td>17 mm</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>16 mm</td>
<td>12 mm</td>
<td>15 mm</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d.: not detected

From Table 1, the 8-hydroxyquinoline 1 and the compounds 3 and 5 exhibited interesting antibacterial activity, where inhibition diameters between 20 mm and 30 mm for the compound 3, and between 13 mm and 25 mm for the compound 5, this activity is generally less than those of control antibiotics tetracycline and streptomycin, they presented successively an inhibition diameter between 13 mm and 20 mm and between 12 mm and 16 mm. On the other hand, the compounds 2.c and 4 showed activity from which the inhibition diameters between 3 mm and 7 mm for the compound 2.c, and between 3 mm and 10 mm for the compound 4. Compounds 2.a and 2.b did not show any activity against the four bacteria.

The antifungal activity of compounds synthesized from 8-hydroxyquinoline was performed on three plant pathogenic fungi: *Botrytis cinerea*, *Fusarium oxysporum* and *Verticillium albo atrum*. The compounds are tested at the concentration of 50 µg / disk in order to determine the margin of inhibition after an incubation time, so we tested the antibiotic amphotericin B as reference and a positive control. The results obtained are presented in Table 2, from which this table shows the margins of inhibition after 15 days of incubation.
Table 2. Antifungal activity of the compounds derived from 8-hydroxyquinoline, and the amphotericin B.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>B. cinerea</th>
<th>F. oxysporum</th>
<th>V. albo atrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23 mm</td>
<td>29 mm</td>
<td>54 mm</td>
</tr>
<tr>
<td>2.a</td>
<td>11 mm</td>
<td>28 mm</td>
<td>16 mm</td>
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<tr>
<td>2.b</td>
<td>5 mm</td>
<td>10 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>2.c</td>
<td>6 mm</td>
<td>25 mm</td>
<td>5 mm</td>
</tr>
<tr>
<td>3</td>
<td>15 mm</td>
<td>17 mm</td>
<td>23 mm</td>
</tr>
<tr>
<td>4</td>
<td>5 mm</td>
<td>9 mm</td>
<td>10 mm</td>
</tr>
<tr>
<td>5</td>
<td>20 mm</td>
<td>17 mm</td>
<td>21 mm</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>22 mm</td>
<td>13 mm</td>
<td>15 mm</td>
</tr>
</tbody>
</table>

The results obtained during this antifungal activity showed that compounds 2.a, 2.c, 3 and 5 have presented an interesting activity, and are more active than the positive control, particularly V. albo atrum. Secondly, other compounds gave an average activity against all three fungi.

The cytotoxicity test was performed according to the method against Brine shrimp larvae shrimp Artemia salina. The compounds obtained in synthesis were tested, and the two products are used as positive controls: potassium dichromate and podophyllotoxin. The results obtained are shown in Table 3.

Table 3. Results of cytotoxicity test.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LD50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>2.a</td>
<td>9.5</td>
</tr>
<tr>
<td>2.b</td>
<td>51.2</td>
</tr>
<tr>
<td>2.c</td>
<td>153.7</td>
</tr>
<tr>
<td>3</td>
<td>28.5</td>
</tr>
<tr>
<td>4</td>
<td>116.1</td>
</tr>
<tr>
<td>5</td>
<td>33.5</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>35.7</td>
</tr>
<tr>
<td>Podophyllotoxin</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The results obtained in this test showed that the compounds 1, 2.a, 3 and 5 have significant cytotoxicity against the larvae of Artemia salina, where the lethal doses 50 were respectively 2.9, 9.5, 28.5 and 33.5 µg / ml. Compounds 2.b, 2.c and 4 are presented low toxicity relative to reference products, and they were respectively lethal doses 50: 51.2, 153.7 and 116.1 µg / ml.

4. Conclusion:
This work led to the development of syntheses for new alkaloids containing the 8-hydroxyquinoline including new methods of skeletal fixation on different starting reagents for 6 new alkaloids. These compounds obtained of 8-hydroxyquinoline derivatives demonstrated in vitro antimicrobial properties and remarkable toxicite, which will provide new opportunities for the use in various biological applications.

References:


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