Antibacterial activity of coumarine derivatives synthesized from 8-amino-4,7-dihydroxy-chromen-2-one and comparison with standard drug

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ABSTRACT

In present paper, we report the organic syntheses of four compounds from 8-Amino-4,7-dihydroxy-chromen-2-one and describe the results of antibacterial activity of purified compounds. Compounds 3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one (1a), 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl]-4,7-dihydroxy-chromen-2-one (2a), 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl]-7-hydroxy-chromen-2-one (3a), 8-[bis-(2-chloro-ethyl)-amino]-2-chloro-7-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione (4a), have been synthesized and characterized using melting points, IR spectra, ¹H-NMR and ¹³C-NMR spectra. The antibacterial activity of synthesized compounds and streptomycin and cefalexine at concentrations of 2mg/ml, 3mg/ml and 5mg/ml, have been evaluated against three strains of bacterial culture; Staphylococcus aureus, E.coli and Bacillus cereus. The compounds show bacteriostatic and bactericidal activity.

Keywords: Coumarine derivatives, antibacterial activity, IR, ¹H-NMR, ¹³C-NMR, Streptomycin.

INTRODUCTION

Starting from 8-Amino-4,7-dihydroxy-chromen-2-one (a); derivatives (1a,2a,3a,4a) are synthesized. Coumarin derivatives are large group of heterocyclic with oxygen as heteroatom. Coumarin is a chemical compound (specifically, a benzo-α-pyrene) found in many plants notably in high concentration in the tonka bean (Dipteryx odorata), vanilla grass (Anthoxanthum odoratum), woodruff (Galium odoratum), mullein (Verbascum spp), and sweet grass (Hierochloe odorata). Coumarine and their derivatives have shown various biological activities. Their fame has come mainly from their antithrombic, antiinflammatory, vasodilatory, and antiviral activities. Other several coumarin derivatives have antimicrobial properties (Sanghyun; et al 1996; Mohareb et al 2007; Nofal et al 2000), with reflux and condensation we have synthesized some new coumarin derivatives and to investigate their antibacterial activity against Staphylococcus aureus, E.coli and Bacillus cereus. The antibacterial activity of synthesized compounds is compared with antibacterial activity of Cefalexine and Streptomycine.

EXPERIMENTAL SECTION

Experimental Chemistry
Compounds 3-Acetyl-8 - amino -4,7- dihydroxy-chromen-2-one (1a), 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) - ethyl]-4,7- dihydroxy-chromen-2-one (2a), 8-Amino-4-[bis-(2-chloro-ethyl)-
amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxy – chromen-2-one (3a), 8-[Bis-(2-chloro-ethyl)-amino] -2-chloro -7- [1-(6- chloro-4- hydroxy -4,5- dihydro – pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxo-4-aza-phenanthrene-3,6-dione (4a) are synthesized.

**Measurement**

The identification of 8-Amino-4,7-dihydroxy-chromen-2-one derivatives (1a, 2a, 3a, 4a), is made by using melting point, IR, $^1$H NMR, $^{13}$C NMR spectra and elemental analysis. Melting point was determined on a Electrothermal apparatus (Fisher Scientific 2555) in a open capillary tube and are uncorrected. Infrared spectra were recorded in cm$^{-1}$ for KBr pellets on a FT-IR Shimadzu 8400S spectrophotometer with resolution 4 cm$^{-1}$. $^1$H NMR spectra were recorded on a Bruker UNITY plus-500 ‘NMR 1’ spectrometer using DMSO-d$_6$ as the solvent and TMS as the internal references standard ($\sigma = 0,00$ ppm). Chemical shifts are expressed in $\delta$ ppm. Mass spectra were taken on a LKB 9000 mass spectrometer.

Element analysis was performed on a Perkin-Elmer 240 BCHN analyzer. The purity of the compounds (synthesized) was routinely checked by TLC using Merck Kieselgel-60 (F-254) and benzene, toluene, glacial acetic acid (80:10:10) as mobile phase.

The spots were exposed in iodine vapour for visualization.

**2.2: Preparation of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one (1a)**

For this synthesis is used as substrat 8-Amino-4,7-dihydroxy-chromen-2-one in a 100 ml flask mixed 3 g of 8-Amino-4,7-dihydroxy – chromen -2-one with 6ml CH$_3$COOH, and 1,5 ml POCl$_3$. The mixture was refluxed at 250°C for ca. 90 min. The obtained crystals brown and white are filtered and rinsed with ethanol and dried at room temperature. Recrystallization from absolute ethanol gave a white product of 80% yield, melting point 129°C.

**Scheme 1. Synthesis of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one (1a)**

**2.3: Preparation of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) -ethyl]-4,7- dihydroxy-chromen-2-one (2a)**

In a 100 ml flask were mixed 2.5g of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one, 2g 2-Amino-6-chloro-4,5-dihydropyridin-4-ol with 5ml C$_2$H$_5$OH, 0,3 ml Et$_3$N. The mixture was refluxed at 80°C for ca. 9h. The obtained yellow crystals are filtered and dried at room temperature. Recrystallization form C$_2$H$_5$OH gave yellow crystals product of 70% yield, meltingpoint, 245°C. (Scheme 2).

**2.4: Preparation of 8-Amino-4-[bis-(2-chloro-ethyl) -amino] -3-[1 -(6- chloro-4- hydroxy-4,5-dihydro-pyrimidin-2 -ylimino) -ethyl] -7- hydroxy – chromen-2-one (3a)**

In a 100 ml flask were mixed 1.5g of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) -ethyl]-4,7- dihydroxy-chromen-2-one, 1g Bis-(2-chloro-ethyl)-amine, with 5 ml CH$_3$CN and 0,2 ml Et$_3$N as katalyzer. The mixture was refluxed at 95°C in water bath for ca. 22 h. The flask was placed in an ice bath for 1h until yellow crystalline precipitate was formed. After filtration the product was recrystallized from CH$_3$CN. The recrystallization gave a yellow product at 70% yield, melting point: 180°C. (Scheme 3).
Scheme 2 Synthesis of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4,7-dihydroxy-chromen-2-one (2a)

Scheme 3 Synthesis of 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-7-hydroxyl-chromen-2-one (3a)

2.5: Preparation of 8-[Bis-(2-chloro-ethyl)-amino]-2-chloro-7-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione (4a)

In a 100 ml flask were mixed 1g 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-7-hydroxyl-chromen-2-one, 0.7ml Dichloro-acetyl chloride, 4ml Dioxane, 0.1ml Et$_3$N. The mixture was refluxed at 95 °C in water bath for ca. 2 h. The obtained red crystals are filtered and rinsed with C$_2$H$_5$OH and dried at room temperature. Recrystallization from ethanol gave a red product at 60 % yield, melting point 204 °C. (Scheme 4)
2.6: Antibacterial activities

The antibacterial activities of all the compounds were studied against gram-positive bacteria (Staphylococcus aureus and Bacillus c.) and gram-negative bacteria (E.coli).

The disc was watted with N,N-DMF solutions of the synthesized compounds with contreaction 2mg/ml,3mg/ml, and 5mg/ml and then are placed in petridish (d=15cm). The old subculture E.coli and B.cereus were poured and spread in petridish in Agar-Mc-Conkey while S.aureus in Agar-maltoze. The disc were incubated at 35°C for 48h, the control was also maintained with DMF, cefalexin and streptomycin in similar manner and the zones of inhibition of the bacterial growth were measured in mm and the results are summarized in tables.

### Table 2 Antibacterial activity- Staphylococcus aureus

<table>
<thead>
<tr>
<th>Compound</th>
<th>2mg/ml</th>
<th>3mg/ml</th>
<th>5mg/ml</th>
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<tbody>
<tr>
<td>1a</td>
<td>13</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>2a</td>
<td>14</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>3a</td>
<td>15</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>4a</td>
<td>14</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Cephalaxine</td>
<td>9</td>
<td>9</td>
<td>10 µg</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>20</td>
<td>20</td>
<td>10 µg</td>
</tr>
</tbody>
</table>
Table 3 Antibacterial activity – E.Coli

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
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<tr>
<td></td>
<td>2mg/ml</td>
</tr>
<tr>
<td>1a</td>
<td>10</td>
</tr>
<tr>
<td>2a</td>
<td>11</td>
</tr>
<tr>
<td>3a</td>
<td>12</td>
</tr>
<tr>
<td>4a</td>
<td>11</td>
</tr>
<tr>
<td>Cephalexine</td>
<td>9</td>
</tr>
<tr>
<td>Streptomycine</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 4 Antibacterial activity – Bacillus cereus

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
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<tbody>
<tr>
<td></td>
<td>2mg/ml</td>
</tr>
<tr>
<td>1a</td>
<td>9</td>
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<tr>
<td>2a</td>
<td>10</td>
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<td>13</td>
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<td>11</td>
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<tr>
<td>Cephalexine</td>
<td>9</td>
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<tr>
<td>Streptomycine</td>
<td>23</td>
</tr>
</tbody>
</table>

The purified synthesized compounds (1a,2a,3a,4a) was subjected to test in vitro its antibacterial activity against three bacterial cultures; Staphylococcus aureus, E.Coli and B.cereus. Antibacterial activity of compounds was investigated applying the Kirby-Bayer method or disc method (d=5.5 mm max. capacity 10 µg).

RESULTS AND DISCUSSION

By reacting equimolar amounts of 8-Amino-4,7-dihydroxy-chromen-2-one and corresponding reagents (according scheme 1) under reflux reaction conditions product 1a is synthesized in 80% yield.

By reacting equimolar amounts of 3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one and corresponding reagents (according scheme 2) under reflux reaction conditions product 2a is synthesized in 70% yield.

By reacting equimolar amounts of 8-Amino-3-[1 -(6 – chloro-4 –hydroxy -4,5- dihydro - pyrimidin- 2- ylimino) - ethyl] -4,7- dihydroxy-chromen – 2 - one and corresponding reagents (according scheme 3) under reflux reaction conditions product 3a is synthesized in 70% yield.

By reacting equimolar amounts 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-di hydro -pyrimidin-2-ylimino) -ethyl]-7-hydroxy1 – chromen–2-one and corresponding reagents (according scheme 4) under reflux reaction conditions product 4a is synthesized in 60% yield.

The structure of 8-Amino-4,7-dihydroxy-chromen-2-one derivatives (1a,2a,3a,4a) were determined from their IR, 1H NMR, 13C NMR spectar and their melting points as follows.

3.1: For (1a); IR bands (KBr, cm⁻¹): 3470(O-H vibration); 3350(N-H); 2850(C-H, alif); 3060(C-H, ar), 1740(C=O), 1600(N-H); 1570(C=C), 1350(N-H); 690(C-H); 649(C-Cl)

3.2: 1H NMR (DMSO-d₆) δ ppm: 0.92 s(3H,CH₃), 4.0d(2H,NH₂), 5.0s(H,OH), 6.4-6.8(2H,ar), 15.0s(H,OH)

3.3: 13C NMR (DMSO): δ ppm: 196(C,C=O), 178(C,COH), 162(C,COO), 138(C,C-O), 126.7(C,N), 113(C,ar), 82(C=C), 70(C,OH), 40.7(C,CH₂), 9.8(C,CH₃)

3.1.1: For (2a) IR bands (KBr, cm⁻¹): 3480(OH), 3337(N-H), 3070(C-H,ar); 2950(C,H,alif) 1760(C=O), 1680(C=O), 1545(C=O), 1224(C-O), 700(C-H,ar) 614(C-Cl)

3.2.1: 1H NMR (DMSO-d₆) δ ppm: 0.9s(3H,CH₃), 5.0(H,OH), 6.4-6.8(2H,ar), 15.0s(H,OH)

3.3.1: 13C NMR (DMSO) δ ppm: 173(C,COH), 163(C=N), 164(C=N), 164.5(C-Cl), 162(C,COO), 143.5(C-OH), 138(C-O), 126(C-N), 118-121(C,ar), 82(C=C), 70(C,C,OH), 40.7(C,CH₂), 9.8(C,CH₃)
3.1.2: For (3a) IR bands (KBr, cm\(^{-1}\))
3490 (OH); 3418 (OH); 3325 (NH); 3050 (C-H, ar); 2922 (C-H, al if);
1750 (C=O); 1655 (C-N); 1644 (C=O); 1530 (C=C); 1248 (C-N); 1 222 (C-O); 690 (C-H, ar); 580 (C-Cl); 543 (C-Cl)

3.2.2: \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm
\(0.93\) s (3H, CH\(_3\)); 1.5 d (H, CH\(_2\)); 2.0 d (H, OH); 2.83 d (4H, 2CH\(_2\)N), 3.50 d (4H, 2CH\(_2\)Cl); 4.0 d (H, NH); 5.0 d (H, OH), 6.4-6.8 (2H, ar)

3.3.2: \(^13\)C NMR (DMSO) \(\delta\) ppm
170 (C, C-N); 164 (C, C-CN); 162.5 (C, COO); 143 (C, C-OH) ;138 (C, C-O); 70 (C, C-CH\(_3\)); 45.1 (C, CH\(_2\)Cl), 40.7 (C, CH\(_2\); 9.5 (C, CH\(_3\))

3.1.3: For (4a) IR bands (KBr, cm\(^{-1}\))
3440 (OH); 3390 (NHCO); 3035 (C-H, ar); 2890 (C-H, alif), 1 760 (C=O), 1680 (C=N); 1533 (C=O), 1230 (C-N); 1215 (C-O) 700 (C-H, ar); 570 (C-Cl), 542 (C-Cl)

3.2.3: \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm
\(0.9\) s (3H, CH\(_3\)); 1.5 d (2H, CH\(_2\)); 2.0 t (H, OH); 2.83 t (2H, CH\(_2\)N); 6.62 s (H, CH); 6.7-7.3 d (2H, ar).8.0 s (H, NHCO)

3.3.3: \(^13\)C NMR (DMSO) \(\delta\) ppm
170 (C, C-N); 164 (C, C=N); 164 (C, C-Cl); 163 (C, CONH); 153.5 (C, C-O); 119 (C, C- N);111,118,120 (3C, ar); 85 (C, C-C=OH), 53 (C, C-N); 45 (C, CH\(_2\)Cl), 40.7 (C, CH\(_2\)); 9.0 (C, CH\(_3\))

CONCLUSION

From the results the followin conclusion were drawn: The study provides the first evidence that compounds (1a,2a,3a,4a) obviously inhibit the growth of S.aureus, E.coli and B.cereus.

The compounds (1a,2a,3a,4a) compared with the antibacterial activity of Streptomycine in S.aureus, E.coli and B.cereus.

This study provided the first evidence that these compounds showed a significant antibacterial effect against S.aureus, E.coli and B.cereus.

The chemical structures of synthesizen compounds were determined according to extensive NMR experiments and published data.

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REFERENCES