Synthesis and Characterization of Some New Azo Dyes Derivatives Via Chalcone and Study Some of Their Biological Activity

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Abstract:
This work includes synthesis of new six membered heterocyclic rings with effective amino group using the reaction of benzylideneacetophenone (chalcone) (1) with thiourea or urea in alcoholic basic medium to form: 1,3-thiazen-2-amine (2), and 1,3-oxazin-2-amine (8) respectively. The diazotization reaction was carried out with sodium nitrite in presence of hydrochloric acid to form diazonium salts which suffered coupling reaction with naphthols and phenols in the presence of sodium hydroxide to form colored azo dyes (4-7, and 10-13). O-methylation reaction of compounds (7) and (10) yielded : 1,3-thiazin-2-yl-diazenyl (14), and 1,3-oxazin-2-yl-diazenyl (15) respectively. The new compounds were characterized using various physical techniques like: UV-Vis., FT-IR, and 1HNMR spectra. Some new compounds were tested against bacteria.

Key words: Heterocyclic, Azo dyes, Benzylideneacetophenone, Thiazine, Oxazine.

Introduction:
Chalcone is an α,β-unsaturated ketone, produced from aldol condensation reaction of acetophenone with benzaldehyde or substituted benzaldehyde in presence of sodium hydroxide as a catalyst as shown below [1-4]:

\[
\text{C}_6\text{H}_5\text{CH} = \text{CHCOPh} \rightarrow \text{CH}_3\text{COPh} + \text{C}_6\text{H}_5\text{CHO}
\]

Its general formula has two isomers cis and trans, trans isomer is more common[1,3]. It has several names such as: benzylideneacetophenone, benzalacetone, and methyl styryl ketone [5]. The name “Chalcone” was given by Kostanecki and Tambor; Chalcones are well known intermediates for synthesizing various heterocyclic compounds [3,4]. Chalcones can be isolated from several plants, and are precursors of flavones compounds, they are similar to enamines, they contain a double bond and prepared from intervention condensation reaction with urea or thiourea and other organic nitrogen compounds to form heterocyclic compounds[3], the reaction should carried out with strong basic conditions and low temperatures to
yield compounds used in pharmaceutical and an industrial purposes[1,6]. When a primary aromatic amine is treated with nitrous acid at low temperature, it will converted to a diazonium salt, the simplest form is benzenediazonium chloride [7,8]. Coupling reaction of azo dye is an organic electrophilic substitution reaction between a diazonium salts and other aromatic compound [8], the products will absorb longer wavelengths of light especially in the visible region compared with the reactants because of the conjugation. Therefore, aromatic azo dyes tend to be brightly colored due to the extended conjugated system[8,9]. Also products of azo coupling reaction used to form pharmaceutical drugs such as sulfa drugs[10,11].

The different application fields of synthetic azo compounds are widely such as foods, medicines, cosmetics, paints, shipbuilding, automobile, plastics, industry, cable manufacture, etc. [12,13]. However, the traditional application field of the synthetic azo dyes still remains the textile industry, and the finishing of fibrous materials in order to impart simultaneously with coloration[6,13]. Using gold (Au) nanoparticles supported on TiO₂ as a catalyst in the aerobic oxidation of aromatic anilines to aromatic azo compounds, also using Au as a hydrogenation catalyst on TiO₂ making it possible to prepare azo compounds directly from nitro aromatics through a two-step (hydrogenation followed by aerobic oxidation), one-pot, and one-catalyst reaction. In addition, the catalytic process is efficient for the synthesis of symmetric and a range of asymmetric aromatic azo compounds from the mixtures of two anilines substituted with electron-donor and electron-acceptor constituents [14].

The aim of this work is to prepare and characterize new series of azo compounds starting from α, β-unsaturated ketone which it were expected to have a biological activity.

Material and Methods:
All chemicals were used through this work purchased from Fluka, Merck Companies and were used without further purifications.

Melting points were recorded using a measuring device melting point type: melting point (SMP 30) Stuart and were uncorrected. Thin layer chromatography (T.L.C.) was carried out using Fertigfollen precoated sheet type: Polygram silica- gel as stationary phase, ethyl acetate as eluent, and the spots were developed with iodine vapor. U.V-Vis. spectra were recorded with spectrophotometer type: SHIMADZU UV spectrophotometer - 1800 using DMSO as a solvent. Infrared spectra were recorded using Fourier transform infrared SHIMADZU(8300) (FT.IR) infrared spectrophotometer by KBr disc. ¹HNMR spectra were recorded on Fourier Transform Varian spectrophotometer, operating at 300 MHz with tetramethylsilane as internal standard, measurements was made at Chemistry Department, AL-AL-BAYT University, Jordan. The biological activity was performed by Biology Department, College of Science, Baghdad University.

Synthesis of 4,6-diphenyl-2H-1,3-thiazin-2-amine(2)[6]
Benzyldeneacetophenone (0.01mol, 2.08gm) (1) was dissolved in alcoholic sodium hydroxide (20mL, 40%) then thiourea (0.01mol, 0.76 gm) was added to it. The reaction was stirred in an ice bath at (5-10)⁰C for two hours, then added to the appropriate amount of ice water, the mixture was stirred for (1) hour. The mixture was kept in
refrigerator for (24) hours, then filtered. A yellow solid material was collected, m.p (113-115) °C and (2.15gm, 81% yield ).

**Synthesis of E-4-((4,6-diphenyl-2H-1,3-thiazin-2-yl)diazenyl)naphthalene-1-ol(4) [15,16]**

Compound (2) (0.65mol,1.729gm) was added to a solution of hydrochloric acid (HCl 10mL + water10 mL ) and cooled in ice-salt bath at (0-5) °C with stirring . To this solution a cold sodium nitrite solution (0.448 gm) in water (3mL) was added slowly with stirring at (5) °C for (1) hour to form diazonium salt (3)., 1-Naphthol (0.936gm) was dissolved in diluted sodium hydroxide solution (10%) and cooled in ice-salt bath at (0-5) °C. To this solution diazonium salt (3) was added with stirring at (5) °C. The colored solid crystals were filtered, washed with distilled water, dried , to obtain compound (4), m.p (161-163) °C and (2.73gm, 79% yield ).

Compounds (5,6 and 7) were prepared by same procedure.

**Synthesis of 4,6-diphenyl-2H-1,3oxazin-2-amine(8)**

Chalcone (0.01mol, 2.08gm) (1) was dissolved in alcoholic sodium hydroxide (20 mL, 40%) then urea (0.01mol, 0.6gm.) was added to it. The reaction was stirred in an ice bath at (5-10) °C for two hours, then added to the appropriate amount of ice water, the mixture was stirred for (1) hour. The mixture was kept in refrigerator for (24) hours, then filtered. A yellow solid was collected, m.p (83-85) °C and (2. 5gm, 77% yield ).

**Synthesis of E-4-(4,6-diphenyl-2H-1,3-oxazin-2-yl)diazaporphathalene-1-ol(10) [15,16]**

Compound (8) (0.065mol,1.625gm) was added to a solution of hydrochloric acid (HCl 10mL + water10 mL ) and cooled in ice-salt bath at (0-5) °C with stirring . To this solution a cold sodium nitrite solution (0.448 gm) in water (3mL) was added slowly with stirring at (5) °C for (1) hour to form diazonium salt (9), 2-Naphthol (0.936gm) was dissolved in diluted sodium hydroxide solution (10%) and cooled in ice-salt bath at (0-5) °C. To this solution diazonium salt (9) was added with stirring at (5)°C. The colored solid crystals were filtered, washed with distilled water, dried , to obtain compound (10), m.p (153-155) °C and (2.12gm, 81% yield ).

Compounds (11,12 and 13) were prepared by same procedure.

**Synthesis of E-2-((4-methoxy-3,5-dimethylphenyl)diazenyl)-4,6-diphenyl-2H-1,3-thiazin(14) and E-2-((4-methoxynaphthalene-1-yl)diazenyl)-4,6-diphenyl-2H-1,3-oxazin(15)[15]**

Compound (7) (0.001mol,0.399gm) was dissolved in diluted sodium hydroxide solution (0.1mL , 10%) and cooled in an ice-bath at (10-15) °C with stirring. To this solution dimethylsulfate (0.001mol, 0.126 gm, 0.1 mL) was added drop wise ; Warm the mixture in water bath at (70-80) °C for (2) hours. The brown solid crystals were filtered, washed with water, dried to obtain compound (14), m.p (203-206) °C and (0. 85gm, 61% yield).

Compound (15) was prepared by same procedure, m.p (167-170) °C and (0.97gm, 74% yield ).

**Results and Discussion:**

The compounds (2) and (8) were prepared using the reaction between
benzylideneacetophenone (chalcone) (1) and thiourea or urea in presence of sodium hydroxide solution (40%) as shown in scheme 1:

\[ \text{Scheme- 1} \]

The suggested mechanism is:

FT-IR spectrum of compound (2), disappeared the bands at (1664) cm\(^{-1}\) due to stretching vibration of carbonyl group (C=O), and (1245) cm\(^{-1}\) due to stretching vibration of thion group (C=S).

The moderate bands at (1350) cm\(^{-1}\) and (692) cm\(^{-1}\) are attributed to stretching vibrations of (C-N) and (C-S) respectively; The bands at (3011) cm\(^{-1}\), (2949) cm\(^{-1}\), and (2920) cm\(^{-1}\) are attributed to stretching vibrations of (C-H) alkene, aromatic, and aliphatic respectively.

FT-IR spectrum of compound (8), displays the bands at (1664.57) cm\(^{-1}\), and (1676) cm\(^{-1}\) due to stretching frequency of carbonyl group (C=O) of starting materials. The bands at (1319) cm\(^{-1}\) and (1292) cm\(^{-1}\) are attributed to (C-N) and (C-O) stretching vibration respectively. The bands at (3192) cm\(^{-1}\), (3084) cm\(^{-1}\), and (2945) cm\(^{-1}\) are attributed to stretching vibrations of
(C-H) alkene, aromatic, and aliphatic respectively (table-1)

**Table (1): FTIR spectral data of compound [2] and [8].**

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>v (NH₂)</th>
<th>v (C=O)</th>
<th>v (C=C) alkene</th>
<th>v (C=N)</th>
<th>v (C=S)</th>
<th>v (C=O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2)</td>
<td>Asym. 3441</td>
<td>1626</td>
<td>1574</td>
<td>1350</td>
<td>692</td>
<td>-</td>
</tr>
<tr>
<td>(8)</td>
<td>Asym. 3419</td>
<td>1618</td>
<td>1568</td>
<td>1310</td>
<td>-</td>
<td>1292</td>
</tr>
</tbody>
</table>

Diazotization of compounds (2) and (8) were carried out by reaction with sodium nitrite in presence of hydrochloric acid as shown in scheme-2 [15-18]:

The coupling reactions were carried out by interactions of diazonium salts with naphthols and phenols to yield colored compounds as shown in scheme-3 [15-18]:
4.6-diphenyl-1-2H-1,3-thiazine-2-diazonium su/t

(E)-4-((4,6-diphenyl-2H-1,3-thiazin-2-yldiazenyl)-2,6-dimethylphenol

(E)-3-chloro-4-((4,6-diphenyl-2H-1,3-thiazin-2-yldiazenyl)phenol

(E)-1-((4,6-diphenyl-2H-1,3-diazin-2-yl)diazenyl)naphthalen-2-ol

(E)-4-((4,6-diphenyl-2H-1,3-oxazin-2-yl)diazenyl)-2,6-dimethylphenol

(E)-3-chloro-4-((4,6-diphenyl-2H-1,3-oxazin-2-yldiazenyl)phenol

(E)-1-((4,6-diphenyl-2H-1,3-oxazin-2-yl)diazenyl)naphthalen-2-ol

Scheme- 3
FT-IR spectra of dyes compounds (4),(5),(6),(7),(10),(11),(12) and (13) showed disappearance of the bands at (3400) cm⁻¹ and (3300) cm⁻¹ due to amino group (NH₂) for compounds (2 and 8), and appearance of bands at (1593-1522) cm⁻¹ due to (trans N=N), and at (3465-3329) cm⁻¹ due to (phenolic OH) ; This supports the incidence of coupling reaction successfully.

The bands at (3062) cm⁻¹, (3051) cm⁻¹, (3011) cm⁻¹, (3020) cm⁻¹, (3045) cm⁻¹, (3047) cm⁻¹, (3086) cm⁻¹, and (3064) cm⁻¹ are attributed to (C-H) aromatic stretching ; The bands at (980) cm⁻¹ and (994) cm⁻¹ attributed to (C-Cl) aromatic stretching respectively for compounds (6 and 12).

FT-IR spectrum of compound (5), showed absorption band at lower frequency due to intramolecular hydrogen bonding between acidic phenol proton and electronic pair of azo group nitrogen [19] (table-2).

Compounds (7) and (10) can be methylated readily with dimethyl sulfate (DMS) forming ethers (14) and (15) as shown in scheme-4 [15].
The suggested mechanism is:

$$\text{R} = \text{Ph} \quad \text{N} \quad \text{Me}$$

$$\text{OH}$$

$$\text{OH}$$

$$(7)$$

$$\text{Me}$$

$$(10)$$

$$(14)$$

$$(15)$$

$$_\text{CH}_3\text{NaSO}_4$$

The FT-IR spectrum of compound (14), showed disappearance of (OH) band at (3404) cm\(^{-1}\), and appearance the band at (1193) cm\(^{-1}\) attributed to (C-O) ether group. The bands at (3045.6) cm\(^{-1}\), (2972) cm\(^{-1}\), and (2987.7) cm\(^{-1}\) are attributed to (C-H) aromatic stretching, (C-H) aliphatic stretching and (C-H) alkene stretching respectively (table-3).

The FT-IR spectrum of compound (15), showed disappearance of (OH) band at (3386) cm\(^{-1}\), and displays the band at (1170) cm\(^{-1}\) attributed to (C-O) ether group. The bands at (3050) cm\(^{-1}\), (2993), (2931) cm\(^{-1}\), and (3012) cm\(^{-1}\) are attributed to (C-H) aromatic stretching, (C-H) aliphatic stretching, and (C-H) alkene stretching respectively (table-3).

Table (2): FT-IR spectral data of compounds (4), (5), (6), (7), (10), (11), (12) and (13).

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>- (O-H) phenolic</th>
<th>- (C=C) aromatic</th>
<th>- (C=O) aldehyde</th>
<th>- (C=N) stretching</th>
<th>- (C-N) transesterification</th>
<th>- (C-S)</th>
<th>- (C=O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4) 3449</td>
<td>1649 &amp; 1449</td>
<td>166</td>
<td>167</td>
<td>157</td>
<td>97</td>
<td>68</td>
<td>9</td>
</tr>
<tr>
<td>(5) 3465</td>
<td>1630 &amp; 1467</td>
<td>168</td>
<td>169</td>
<td>157</td>
<td>98</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>(6) 3464</td>
<td>1668 &amp; 1458</td>
<td>169</td>
<td>172</td>
<td>157</td>
<td>96</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>(7) 3404</td>
<td>1576 &amp; 1436</td>
<td>162</td>
<td>161</td>
<td>157</td>
<td>98</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>(10) 3386</td>
<td>1443 &amp; 1437</td>
<td>161</td>
<td>161</td>
<td>159</td>
<td>12</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>(11) 3360</td>
<td>1439 &amp; 1383</td>
<td>160</td>
<td>167</td>
<td>153</td>
<td>12</td>
<td>99</td>
<td>25</td>
</tr>
<tr>
<td>(12) 3329</td>
<td>1499 &amp; 1437</td>
<td>161</td>
<td>167</td>
<td>158</td>
<td>12</td>
<td>98</td>
<td>25</td>
</tr>
<tr>
<td>(13) 3387</td>
<td>1482 &amp; 1420</td>
<td>162</td>
<td>167</td>
<td>152</td>
<td>13</td>
<td>00</td>
<td>13</td>
</tr>
</tbody>
</table>
Table (3): FT-IR spectral data of compounds [14] and [15].

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>( \text{v}<em>1) (C(</em>\text{C}) aromat e)</th>
<th>( \text{v}<em>2) (C(</em>\text{C}) alt. e)</th>
<th>( \text{v}<em>3) (C(</em>\text{O})) trans</th>
<th>( \text{v}_4) (N=(\text{N})) trans</th>
<th>( \text{v}<em>5) (C(</em>\text{N}))</th>
<th>( \text{v}_6) C=S</th>
<th>( \text{v}_7) C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>(14)</td>
<td>1562 &amp; 1405</td>
<td>1584</td>
<td>1612</td>
<td>1583</td>
<td>121</td>
<td>119</td>
<td>69</td>
</tr>
<tr>
<td>(15)</td>
<td>1425 &amp; 1387</td>
<td>1603</td>
<td>1713</td>
<td>1584</td>
<td>120</td>
<td>137</td>
<td>-</td>
</tr>
</tbody>
</table>

UV. - Vis. spectra of the prepared compounds showed high intense absorption peaks at the range (310-220) nm which assigned to overlap of \((\pi - \pi^*)\) and \((\text{n} - \pi^*)\) transitions [18,19].

\(^1\)HNMR spectrum of compound (2), showed a singlet signal at \(\delta = 2.503\) ppm attributed to a proton bonded to (C5) in thiazine ring, and singlet signal at \(\delta = 3.348\) ppm due to a proton bonded to (C2) of thiazine ring; while a multiplet signal at \(\delta = 7.191-8.433\) ppm due to interference of amino and phenyl protons.

\(^1\)HNMR spectrum of compound (4), showed a singlet signal at \(\delta = 2.303\) ppm due to proton on (C5) of thiazine ring and another singlet signal at \(\delta = 3.341\) ppm due to proton on (C2) of thiazine ring; A multiplet signal at \(\delta = 7.452 - 8.167\) ppm due to naphtholic proton interference with phenyl protons.

\(^1\)HNMR spectrum of compound (8), showed a singlet signal at \(\delta = 2.501\) ppm attributed to a proton bonded to (C5) in oxazine ring, and singlet signal at \(\delta = 3.341\) ppm due to a proton bonded to (C2) of oxazine ring; while a multiplet signal at \(\delta = 7.00 - 7.88\) ppm due to interference of amino and phenyl protons.

\(^1\)HNMR spectrum of compound (12), showed a singlet signal at \(\delta = 2.156\) ppm due to proton on (C5) of oxazine ring and another singlet signal at \(\delta = 3.301\) ppm due to proton on (C2) of oxazine ring; A multiplet signal at \(\delta = 7.436-9.118\) ppm due to interference of phenolic proton and phenyl protons.

Table (5) showed the physical properties of the prepared compounds.

Table (5): Physical constants of the prepared compounds.

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>M.p. °C</th>
<th>Yield %</th>
<th>Color</th>
<th>Recryst. solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>113 - 115</td>
<td>75</td>
<td>Yellow</td>
<td>EtOH</td>
</tr>
<tr>
<td>4</td>
<td>161 - 163</td>
<td>70</td>
<td>Yellow</td>
<td>EtOH</td>
</tr>
<tr>
<td>5</td>
<td>151 - 154</td>
<td>80</td>
<td>Yellow</td>
<td>EtOH</td>
</tr>
<tr>
<td>6</td>
<td>175 - 176</td>
<td>70</td>
<td>Deep brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>7</td>
<td>191 - 193</td>
<td>65</td>
<td>Reddish brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>8</td>
<td>83 - 85</td>
<td>73</td>
<td>Deep yellow</td>
<td>EtOH</td>
</tr>
<tr>
<td>10</td>
<td>153 - 155</td>
<td>60</td>
<td>Red - orange</td>
<td>EtOH</td>
</tr>
<tr>
<td>11</td>
<td>147 - 149</td>
<td>61</td>
<td>Red - brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>12</td>
<td>156 - 157</td>
<td>67</td>
<td>Deep brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>13</td>
<td>179 - 181</td>
<td>73</td>
<td>Brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>14</td>
<td>203 - 205</td>
<td>60</td>
<td>Brown</td>
<td>EtOH</td>
</tr>
<tr>
<td>15</td>
<td>167 - 170</td>
<td>65</td>
<td>Light brown</td>
<td>EtOH</td>
</tr>
</tbody>
</table>

Antibacterial activity

In this work, the antibacterial test was performed according to the disc diffusion method. Compounds (4, 12 and 14) were assayed for their antimicrobial activity \textit{in vitro} against Gram-negative bacteria (\textit{Escherichia Coli}) and Gram- positive bacteria (\textit{staphylococcus aurous}). Prepared agar and Petri dishes were sterilized by autoclaving for 15min. at 121 C\(^0\). The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all 6 mm in diameter. These holes were filled with 0.1 ml of the prepared compounds (20mg of the compound dissolved in 1mL of DMSO solvent), DMSO was used as a solvent. These plates were incubated at 37 \(^0\)C for 24hr for...
bacteria. The inhibition zones caused by the prepared compounds were examined. The results of the preliminary screening tests are listed in (table- 6).

Table (6): Results of antibacterial activity of the tested compounds.

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Escherichia Coli</th>
<th>Klebsiella Pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(12)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(14)</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

- = No inhibition = inactive., + = (5-10) mm = slightly active., ++= (11-20) mm = moderately active.

References:
and the application of chalcone - derivatives in photo- alignment layer of liquid crystal display;


تحضير و تشخيص بعض مشتقات جديدة لأصبغ الأزور من جالكون و دراسة بعض الفعالية البيولوجية لهم

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الخلاصة:
تضمن البحث تحضير حلقات جديدة غير متجانسة سداسية معوضة بمجموعة الأمين الفعالة وذلك عن طريق تفاعل بنزيلينين أسيتوفيون (جالكون) (1) مع الثأثويريا أو البوريا في وسط قاديي كحولي و تكوي: 3,1-ثابازين-2-أمين (2) و 3,1- أوكسازين-2- أمين (8) على التوالي, ثم تحضير ملح الديازونيوم بمقها (2) و (8) مع نتريت الصوديوم يوجد حامض الهيدروكلاوريك لتكوين المركبات (3) و (9) التي تعاني تفاعل ازدواج مع الفينولات و النفلولات بوجود القاعدة لتكوين الأصبغ الملونة (4-7 و 10-13).
شخصت جميع المركبات الجديدة المحضرة ببطائفة الأصبغة فوق البنفسجية و الأصبغ تحت الحمراء و الرنين النووي المغناطيسي. أتبعت بعض هذه المركبات ضد أنواع من البكتريا.

الكلمات المفتاحية: مركبات حلقية غير متجانسة ، صبغات الأزور ، بنزيلينين أسيتوفيون ، ثابازين ، أوكسازين.