Synthesis, characterization and antibacterial activity of various substituted oxadiazolylpyrazolinyl/isoxazolinylcoumarin derivatives.

Hemlata Kaur, Sunil Kumar, Indu Singh and Ashok Kumar*

ABSTRACT

A series of 7-un/substituted-2-spiro-[5-((1-acetyl-5-(substitutedphenyl)amino)-3-(1-acetyl-5-(substitutedphenyl))pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins (4a-4h) and 7-un/substituted-2-spiro-2-[(-5-(substitutedphenyl)amino-4-(5-(substitutedphenyl))pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins (5a-5h) by the reaction of 7-un/substituted-2-spiro-(3-substitutedarylidinyl chalconyl)-5-(substitutedarylidinylaminochalconyl)-oxadiazol-2-yl)coumarins (3a-3h) with hydrazine hydrate and hydroxyl amine respectively. All the newly synthesized compounds were screened for their antibacterial activity against K. pneumoniae, S. aureus, E. coli and B. sublitis and were compared with the standard drug ciprofloxacin. The most potent antibacterial compound of this series was 4g. Structure of all the compounds were established by the elemental (C, H, N) and spectral (IR, 1H NMR and mass) analysis.

Keywords: Substitutedcoumarin, Oxadiazolylcoumarins, Pyrazolinylcoumarins, Isoxazolinylcoumarins, Antibacterial activity, Acute toxicity.

INTRODUCTION

Coumarin is versatile pharmacophore which exhibits a wide variety of biological activities like antibacterial [1-3] and antimicrobial [4]. Moreover, various organic compounds containing a five membered heterocyclic ring i.e. oxadiazole make up a broad class that attracted attention in the past few years owing to its wide range of biological activities especially antimicrobial [5,6], antibacterial [7], antifungal [7], and anticonvulsant [8] and anti-inflammatory [9] activities. It is interesting to note from chemical literature that various pyrazoline and isoxazoline derivatives were also found to possess wide spectrum of bactericidal, fungicidal, and antimicrobial activities [10-13]. In the light of above observations we report herein the synthesis of compounds (4a-4h) and (5a-5h) with the hope to get better antibacterial agents.

MATERIAL AND METHODS

Antibacterial activity: All the newly synthesized compounds 3a-3h, 4a-4h and 5a-5h were tested for their antibacterial activity. Antibacterial activity was determined by agar cup plate method [14] at a concentration of 100mg/ml using DMF as a solvent against the following organism- Escherichia coli, Staph. Aureus, Klebsiella pneumoniae and B. sublitis. The zone of inhibition of each strain was recorded. The activity has been compared with known standard drug ciprofloxacin at 10 μg/ml concentration. The biological results were analysed statistically by student’s t test. Propylene glycol treated group served as control.

Approximate lethal dose (LD50): The LD50 was determined by the method of Smith [15].

RESULTS AND DISCUSSION

All the newly synthesized compounds 3a-3h, 4a-4h and 5a-5h were screened for their antibacterial activity. The pharmacological data of all the compounds have been reported in table 1.
Table 1: Antibacterial activity of compounds 3a-3h, 4a-4h and 5a-5h.

<table>
<thead>
<tr>
<th>Com. No.</th>
<th>R</th>
<th>R'</th>
<th>Zone of inhibition (diameter in mm)</th>
<th>LD_{50} mg/kg i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>K. Pneumoniae</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>-</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>3a.</td>
<td>H</td>
<td>2-OH</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>3b.</td>
<td>H</td>
<td>4-OCH₃</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>3c.</td>
<td>H</td>
<td>4-OH,3-OCH₃</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>3d.</td>
<td>H</td>
<td>4-N(CH₃)₂</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>3e.</td>
<td>OCH₃</td>
<td>2-OH</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>3f.</td>
<td>OCH₃</td>
<td>4-OCH₃</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>3g.</td>
<td>OCH₃</td>
<td>4-OH,3-OCH₃</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>3h.</td>
<td>OCH₃</td>
<td>4-N(CH₃)₂</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>4a.</td>
<td>H</td>
<td>2-OH</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>4b.</td>
<td>H</td>
<td>4-OCH₃</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>4c.</td>
<td>H</td>
<td>4-OH,3-OCH₃</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>4d.</td>
<td>H</td>
<td>4-N(CH₃)₂</td>
<td>18</td>
<td>17</td>
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<tr>
<td>4e.</td>
<td>OCH₃</td>
<td>2-OH</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>4f.</td>
<td>OCH₃</td>
<td>4-OCH₃</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>4g.</td>
<td>OCH₃</td>
<td>4-OH,3-OCH₃</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>4h.</td>
<td>OCH₃</td>
<td>4-N(CH₃)₂</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>5a.</td>
<td>H</td>
<td>2-OH</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>5b.</td>
<td>H</td>
<td>4-OCH₃</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>5c.</td>
<td>H</td>
<td>4-OH,3-OCH₃</td>
<td>16</td>
<td>-</td>
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<tr>
<td>5d.</td>
<td>H</td>
<td>4-N(CH₃)₂</td>
<td>20</td>
<td>21</td>
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<tr>
<td>5e.</td>
<td>OCH₃</td>
<td>2-OH</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>5f.</td>
<td>OCH₃</td>
<td>4-OCH₃</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>5g.</td>
<td>OCH₃</td>
<td>4-OH,3-OCH₃</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>5h.</td>
<td>OCH₃</td>
<td>4-N(CH₃)₂</td>
<td>22</td>
<td>17</td>
</tr>
</tbody>
</table>

The characteristic feature of this series is the presence of different heterocyclic moieties at 2\textsuperscript{nd} position of substituted coumarin ring. Compound 3a showed mild antibacterial activity against E. coli and B. Sublitis. This compound was devoid of antibacterial activity against K. pneumoniae and S. aureus. Compounds 3b, 3c and 3f exhibited moderate...
activity against S. aureus, E. coli, and B. sublitis. Moreover, compound 3g (having 4-hydroxy-3-methoxyphenyl moiety) showed good activity against E. coli, whereas compound 3h (having NN'-dimethylaminophenyl moiety) showed good activity against S. aureus (20 mm zone of inhibition). Furthermore, in the next step compounds (various substituted pyrazoline derivatives), an increase in antibacterial activity was noticed. Compounds 4a showed significant antibacterial activity against K. pneumoniae. Moreover, compounds 4c, 4f and 4h elicited good antibacterial response against K. pneumoniae, E. coli and B. sublitis (i.e. 19-24 mm zone of inhibition) and moderate activity against S. aureus. Furthermore, compound 4g namely 7-methoxy-3-spiro-[2-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-4-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl))pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin has shown higher antibacterial activity (i.e. zone of inhibition 28, 24, 27, and 22mm against K. pneumoniae, S. aureus, E. coli, and B. sublitis respectively) than standard drug ciprofloxacin. On the other side, i.e. compounds 5a-5h (having various substituted isoxazoline ring) exhibited different antibacterial responses against different bacterial strain. In these compounds, compound 5d and 5f showed good activity against K. pneumoniae, S. aureus and moderate activity against E. coli and B. sublitis. However, compound 5h exhibited good response against K. pneumoniae. Moreover, compound 5g exhibited equipotent activity against K. pneumoniae, S. aureus and higher activity against E. coli than standard drug ciprofloxacin. The latter compound showed less potent activity against B. sublitis than standard drug ciprofloxacin.

5. Conclusion
From this study, we may concluded that:
1. Compounds having 5-membered pyrazoline ring exhibited better activity than their corresponding isoxazoline derivatives.
2. Compounds with 4-hydroxy-3-methoxyphenyl moiety (i.e. compound 4g and 5g) at 5th position of pyrazoline and isoxazoline ring showed more promising results than the other substituted derivatives against all the bacterial strains.

All reagents and solvents were of analytical grade and used directly. All reagents and solvents were generally used as received from the analytical grade. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. Homogeneity of all the newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G coated plate of 0.5 mm thickness. The eluent was a mixture of different polar and nonpolar solvents in different proportions, and spots were visualized under iodine chamber. Elemental analysis (C, H, N) of all the compounds were performed on CHN analyzer, Carlo Erba 1108 analyzer at the Central Drug Research Institute (Lucknow, India). The IR spectra were recorded on Perkin Elmer 881 FTIR spectrophotometer (\( \nu_{\text{max}} \) in cm\(^{-1} \)). The \(^1\)H-NMR spectra were recorded in CDCl\(_3\) and DMSO\(_d6\) on Bruker DRX-300 FTNMR instrument. Mass spectra were determined on JEOL D-300 instrument.

EXPERIMENTAL PROCEDURE
General procedure for the synthesis of 7-un/substituted-coumarin-2-semicarbazone 1a-1b
A solution of semicarbazide and thiosemicarbazide (2.0 mole) in glacial acetic acid (26 ml) and isatin or indole 2, 3 dione (2.0 mole) and the mixture was refluxed for 3 h. After cooling the solid mass was collected by filtration, washed well with water, dried and recrystallized from appropriate solvent to give compounds 1a and 1b respectively.

**Coumarin-2-semicarbazone 1a:** Yield 85% (Methanol); m.p. 84 °C. IR (KBr, \( \nu_{\text{max}} \) in cm\(^{-1} \)) : 3330 (NH sym.), 3000 (Aromatic C-H str.), 2170 ((C-CN str.), 1690 (C=O str.), 1292 (N-N), 1040 (C-O-C); \(^1\)H-NMR (CDCl\(_3\)) : in ppm 6.82-7.80 (m, 6H, Ar-H), 4.32 (s, 2H, NH\(_2\)), 6.60 (s, 1H, NH). Anal. calcd. for C\(_{10}\)H\(_9\)N\(_3\)O\(_2\): C, 59.11; H, 4.46; N, 20.68: Found : C, 59.10; H, 4.48; N, 20.66%

**7-methoxy-coumarin-2-semicarbazone 1b:** Yield 75% (ethanol); m.p. 90 °C. IR (KBr, \( \nu_{\text{max}} \) in cm\(^{-1} \)) : 3300 (NH sym.), 3010 (Aromatic C-H str.), 2177 ((C-CN str.), 1685 (C=O str.), 1294 (N-N), 1035 (C-O-C); \(^1\)H-NMR (CDCl\(_3\)) : in ppm 3.81 (s, 3H, OCH\(_3\)), 6.92-7.83 (m, 5H, Ar-H), 4.32 (s, 2H, NH\(_2\)), 6.63 (s, 1H, NH). Anal. calcd. for C\(_{11}\)H\(_{11}\)N\(_3\)O\(_3\): C, 56.65; H, 4.75; N, 18.02: Found : C, 56.63; H, 4.70; N, 18.03%
Scheme 1: Synthetic route of coumarin derivatives
General procedure for the synthesis of 7-un/substituted-2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2a-2b

A mixture of compounds 1a and 1b (1.0 mole) and freshly distilled acetic anhydride (40 ml) was heated to 110-120 °C for 4 h and after removal of acetic anhydride from the reaction mixture with the help of rotary vacuum evaporator, a solid mass was obtained which was recrystallized from suitable solvents to give compounds 2a and 2b.

2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2a:
Yield 76% (Acetone); m.p. 111 °C. IR (KBr, max in cm⁻¹): 3330 (NH sym.), 3008 (Aromatic C-H str.), 2178 (-C N str.), 1684 (C=O str.), 1290 (N-N), 1038 (C-O-C); ¹H-NMR (CDCl₃) in ppm 3.47 (s, 6H, 2 x COCH₃), 6.98-7.81 (m, 6H, Ar-H), 8.63 (s, 1H, NH). Anal. calcd. for C₁₄H₁₃N₃O₄: C, 58.53; H, 4.56; N, 14.63: Found : C, 58.50; H, 4.54; N, 14.66%

7-Methoxy-2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2b:
Yield 73% (Methanol); m.p. 120 °C. IR (KBr, max in cm⁻¹): 3430 (OH), 3420 (NH sym.), 3000 (Aromatic C-H str.), 2180 (-CN str.), 1689 (C=O str.), 1295 (N-N), 1037 (C-O-C); ¹H-NMR (CDCl₃+DMSO-d₆) in ppm 2.70 (s, 6H, 2 x COCH₃), 3.52 (s, 3H, OCH₃), 6.88-7.71 (m, 5H, Ar-H), 8.60 (s, 1H, NH). Anal. calcd. for C₁₅H₁₅N₃O₅: C, 56.78; H, 4.76; N, 13.24: Found : C, 56.78; H, 4.76; N, 13.24%

General procedure for the synthesis of 7-un/substituted-2-spiro-(3-substitutedarylidinyl chalconyl)-5-(substituted arylidinylaminochalconyl)-oxadiazol-2-yl)coumarins 3a-3h

A solution of compound 2a-2b (0.5 mol) in absolute ethanol (50 ml) in 2% NaOH and various substituted aromatic aldehydes (0.1 mole) was refluxed for 8-12 h, concentrated, cooled and poured onto ice. The solid thus obtained was filtered, washed with water and recrystallised from appropriate solvent to obtained compounds 3a-3d.

2-spiro-(3-(2-hydroxyarylidinylchalconyl)-5-(2-hydroxyarylidinylaminoxhalconyl)-oxadiazol-2-yl)coumarin 3a:
Yield 80% (ethanol); m.p. 138 °C. IR (KBr, max in cm⁻¹): 3440 (OH), 3368 (NH sym.), 3010 (Aromatic C-H str.), 2195 (-CN str.), 1698 (C=O str.), 1570 (CH=CH), 1292 (N-N), 1045 (C-O-C); ¹H-NMR (CDCl₃) in ppm 6.60 (d, 2H, 2 x COCH), 6.88-7.79 (m, 14H, Ar-H), 8.87 (s, 1H, NH), 8.82 (d, 2H, 2 x =CHAr), 11.21 (s, 2H, 2 x OH). Anal. calcd. for C₂₈H₂₁N₃O₆: C, 67.87; H, 4.27; N, 8.48: Found : C, 67.89; H, 4.24; N, 8.46%

2-spiro-(3-(2-methoxyarylidinylchalconyl)-5-(2-methoxyarylidinylaminoxhalconyl)-oxadiazol-2-yl)coumarin 3b:
Yield 70% (Methanol); m.p. 142 °C. IR (KBr, max in cm⁻¹): 3347 (NH sym.), 3000 (Aromatic C-H str.), 2189 (-CN str.), 1687 (C=O str.), 1577 (CH=CH), 1290 (N-N), 1038 (C-O-C); ¹H-NMR (CDCl₃+DMSO-d₆) in ppm 6.60 (d, 2H, 2 x COCH), 6.78-7.89 (m, 14H, Ar-H), 8.80 (s, 1H, NH), 8.87 (d, 2H, 2 x =CHAr), 3.50 (s, 6H, OCH₃). Anal. calcd. for C₃₀H₂₅N₃O₆: C, 68.82; H, 4.81; N, 8.03: Found : C, 68.80; H, 4.86; N, 8.02%

2-spiro-(3-(4-hydroxy-3-methoxyarylidinylchalconyl)-5-(4-hydroxy-3-methoxyarylidinylaminochalconyl)-oxadiazol-2-yl)coumarin 3c:
Yield 65% (ethanol); m.p. 154 °C. IR (KBr, max in cm⁻¹): 3447 (OH), 3012 (Aromatic C-H str.), 3337 (NH sym.), 2190 (-CN str.), 1683 (C=O str.), 1570 (CH=CH), 1292 (N-N), 1039 (C-O-C); ¹H-NMR (DMSO-d₆) in ppm 6.63 (d, 2H, 2 x COCH), 6.79-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.45 (s, 6H, OCH₃), 11.20 (s, 2H, OH). Anal. calcd. for C₃₀H₂₅N₃O₈: C, 64.86; H, 4.54; N, 7.56: Found : C, 64.83; H, 4.52; N, 7.59%

2-spiro-(3-(4-NN'-dimethylarylidinylchalconyl)-5-(4-NN'-dimethylarylidinylaminochalconyl)-oxadiazol-2-yl)coumarin 3d:
Yield 75% (Petroleum ether); m.p. 149 °C. IR (KBr, max in cm⁻¹): 3341 (NH sym.), 3000 (Aromatic C-H str.), 2189 (-CN str.), 1683 (C=O str.), 1571 (CH=CH), 1292 (N-N), 1037 (C-O-C); ¹H-NMR (CDCl₃) in ppm 1.30 (s, 12H, 2 x N(CH₃)₂), 6.61 (d, 2H, 2 x COCH), 6.64-7.70 (m, 14H, Ar-H), 8.70 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr). Anal. calcd. for C₃₂H₃₁N₅O₄: C, 69.93; H, 5.69; N, 12.74: Found : C, 69.90; H, 5.66; N, 12.78%
6.61 (d, 2H, 2 x COCH), 6.77-7.83 (m, 13H, Ar-H), 8.83 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.53 (s, 9H, 3 x OCH3). Anal. calcd. for C31H27N3O7: C, 67.27; H, 4.92; N, 7.59; Found : C, 67.25; H, 4.94; N, 7.60%

7-Methoxy-2-spiro-(3-(4-hydroxy-3-methoxyarylidinyl)oxadiazol-2-yl)coumarin 3g: Yield 72% (DMF-water); m.p. 179 0C. IR (KBr, max in cm -1): 3452 (OH), 3352 (NH sym.), 3000 (Aromatic C-H str.), 2196 (-CN str.), 1689 (C=O str.), 1582 (CH=CH), 1294 (N-N), 1064 (C-O-C); 1H-NMR (CDCl3) in ppm 6.64 (d, 2H, 2 x COCH), 6.74-7.80 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.46 (s, 9H, 3 x OCH3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for C31H27N3O9: C, 63.59; H, 4.65; N, 7.18; Found : C, 63.59; H, 4.65; N, 7.20%

7-Methoxy-2-spiro-(3-(4-NN'-dimethylarylidinyl)oxadiazol-2-yl)coumarin 3h: Yield 76% (Methanol); m.p. 173 0C. IR (KBr, max in cm-1): 3342 (NH sym.), 3015 (Aromatic C-H str.), 2200 (-CN str.), 1698 (C=O str.), 1579 (CH=CH), 1292 (N-N), 1067 (C-O-C); 1H-NMR (CDCl3) in ppm: 1.32 (s, 12H, 2 x N (CH3)2), 3.55 (s, 3H, OCH3), 6.65 (d, 2H, 2 x COCH), 6.75-7.86 (m, 13H, Ar-H), 8.70 (s, 1H, NH), 8.80 (d, 2H, 2 x =CHAr), 11.22 (s, 1H, OH). Anal. calcd. for C33H33N5O5: C, 68.38; H, 5.74; N, 12.08, Found : C, 68.30; H, 5.76; N, 12.05%

General procedure for the synthesis of 7-un/substituted-2-spiro-[5-(1-acetyl-5-(substitutedphenyl)amino-3-(1-acetyl-5-(substitutedphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins 4a-4h
To a solution of 3a-3h (0.03 mole) in methanol, hydrazine hydrate (99%) (0.03 mole) and few drops of glacial acetic acid were added. The reaction mixture were refluxed for 10 h, distilled and cooled. The separated solid was filtered, washed with water and recrystallised from suitable solvent to furnish compound 4a-4h.

2-spiro-[5-(1-acetyl-5-(2-hydroxyphenyl)amino-3-(1-acetyl-5-(2-hydroxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4a: Yield 75% (ethanol); m.p. 197 0C. IR (KBr, max in cm -1): 3441 (OH), 3356 (NH sym.), 2980 (Aromatic C-H str.), 2210 (-CN str.), 1720 (C=O str.), 1290 (N-N), 1067 (C-O-C); 1H-NMR (CDCl3) in ppm: 3.57 (s, 6H, 2 x COCH3), 3.75 (d, 4H, 2 x CH2 of pyrazoline ring), 5.74 (d, 2H, 2 x CH of pyrazoline ring), 6.76-7.79 (m, 14H, Ar-H), 8.88 (s, 1H, NH), 11.25 (s, 2H, OH). Anal. calcd. for C32H29N7O6: C, 63.25; H, 4.81; N, 16.14; Found : C, 63.23; H, 4.86; N, 16.13%

2-spiro-[5-(1-acetyl-5-(2-methoxyphenyl)amino-3-(1-acetyl-5-(2-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4b: Yield 70% (ethanol); m.p. 208 0C. IR (KBr, max in cm-1): 3337 (NH sym.), 2982 (Aromatic C-H str.), 2212 (-CN str.), 1712 (C=O str.), 1065 (C-O-C); 1H-NMR (CDCl3) in ppm: 3.53 (s, 6H, 2 x COCH3), 3.73 (d, 4H, 2 x CH2 of pyrazoline ring), 5.75 (d, 2H, 2 x CH of pyrazoline ring), 6.97-7.87 (m, 14H, Ar-H), 8.85 (s, 1H, NH), 3.52 (s, 6H, 2 x OCH3). Anal. calcd. for C34H33N7O6: C, 64.24; H, 5.23; N, 15.42; Found : C, 64.24; H, 5.23; N, 15.42%

2-spiro-[5-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-3-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4c: Yield 71% (Acetone); m.p. 228 0C. IR (KBr, max in cm-1): 3450 (OH), 3351 (NH sym.), 2985 (Aromatic C-H str.), 2210 (-CN str.), 1712 (C=O str.), 1290 (N-N), 1067 (C-O-C); 1H-NMR (CDCl3) in ppm: 3.50 (s, 6H, 2 x COCH3), 3.74 (d, 4H, 2 x CH2 of pyrazoline ring), 5.76 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 6H, 3 x OCH3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for C34H33N7O8: C, 61.16; H, 4.98; N, 14.68; Found : C, 61.25; H, 4.81; N, 14.69%

2-spiro-[5-(1-acetyl-5-(4-NN'-dimethylaminophenyl)amino-3-(1-acetyl-5-(4-NN'-dimethylaminophenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4d: Yield 70% (Ethanol); m.p. 212 0C. IR (KBr, max in cm -1): 3344 (NH sym.), 2982 (Aromatic C-H str.), 2212 (-CN str.), 1712 (C=O str.), 1290 (N-N), 1067 (C-O-C); 1H-NMR (CDCl3) in ppm: 1.32 (s, 12H, 2 x N(CH3)2), 3.53 (s, 6H, 2 x COCH3), 3.70 (d, 4H, 2 x CH2 of pyrazoline ring), 5.72 (d, 2H, 2 x CH of pyrazoline ring), 6.75-7.86 (m, 14H, Ar-H), 8.70 (s, 1H, NH). Anal. calcd. for C36H39N9O4: C, 65.34; H, 5.94; N, 19.05; Found : C, 65.37; H, 5.92; N, 19.05%

7-Methoxy-2-spiro-[5-(1-acetyl-5-(2-hydroxyphenyl)amino-3-(1-acetyl-5-(2-hydroxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4e: Yield 73% (Methanol); m.p. 228 0C. IR (KBr, max in cm -1): 3450 (OH), 3351 (NH sym.), 2985 (Aromatic C-H str.), 2210 (-CN str.), 1712 (C=O str.), 1290 (N-N), 1067 (C-O-C); 1H-NMR (CDCl3) in ppm: 3.39 (s, 3H, OCH3), 3.61 (s, 6H, 2 x COCH3), 3.70 (d, 4H, 2 x CH2 of pyrazoline ring), 5.72 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 6H, 3 x OCH3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for C33H31N7O7: C, 62.16; H, 4.90; N, 15.38; Found : C, 62.17 H, 4.90; N, 15.36%
7-Methoxy-2-spiro-[5-(1-acetyl-5-(2-methoxyphenyl)amino-3-(1-acetyl-5-(2-methoxyphenyl)pyrazolin-3-yl)]-1,3,4-oxadiazol-2-yl]coumarin 4f: Yield 70% (ethanol); m.p. 228 °C. IR (KBr, max in cm⁻¹): 3345 (NH sym.), 2990 (Aromatic C-H str.), 1710 (C=O str.), 1046 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 3.56 (s, 6H, 2 x COCH₃), 3.74 (d, 4H, 2 x CH₂ of pyrazoline ring), 5.76 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 13H, Ar-H). Anal. calcd. for C₃₅H₃₅N₇O₇: C, 63.15; H, 5.30; N, 14.73: Found : C, 63.12; H, 5.29; N, 14.76%

7-Methoxy-2-spiro-[5-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-3-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)pyrazolin-3-yl)]-1,3,4-oxadiazol-2-yl]coumarin 4g: Yield 69% (DMF-water); m.p. 240 °C. IR (KBr, max in cm⁻¹): 3440 (OH), 3348 (NH sym.), 2987 (Aromatic C-H str.), 2220 (-CN str.), 1713 (C=O str.), 1292 (N-N), 1048 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 3.49 (s, 6H, 2 x COCH₃), 3.74 (d, 4H, 2 x CH₂ of pyrazoline ring), 5.75 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH₃), 11.18 (s, 2H, 2 x OH). Anal. calcd. for C₃₅H₃₅N₇O₉: C, 60.25; H, 5.06; N, 14.05: Found : C, 60.23; H, 5.07; N, 14.07%

7-Methoxy-2-spiro-[5-(1-acetyl-5-(4-NN'-dimethylaminophenyl)amino-3-(1-acetyl-5-(4-NN'-dimethylaminophenyl)pyrazolin-3-yl)]-1,3,4-oxadiazol-2-yl]coumarin 4h: Yield 71% (Methanol); m.p. 231 °C. IR (KBr, max in cm⁻¹): 3347 (NH sym.), 2992 (Aromatic C-H str.), 2200 (-CN str.), 1714 (C=O str.), 1291 (N-N), 1062 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 1.34 (s, 12H, 2 x N(CH₃)₂), 3.40 (s, 3H, OCH₃), 3.50 (s, 6H, 2 x COCH₃), 3.83 (d, 4H, 2 x CH₂ of pyrazoline ring), 5.77 (d, 2H, 2 x CH of pyrazoline ring), 6.78-7.89 (m, 13H, Ar-H), 8.73 (s, 1H, NH), 11.22 (s, 1H, OH). Anal. calcd. for C₃₇H₄₁N₉O₅: C, 64.24; H, 5.97; N, 18.22: Found : C, 64.27; H, 5.93; N, 18.29%

General procedure for the synthesis of 7-un/substituted-3-spiro-[2-(5-(substitutedphenyl)amino-4-(5-(substitutedphenyl))isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins 5a-5h

To a solution of 3a-3h (0.03 mole) in methanol (50 ml), hydroxyl amine (0.03 mole) was added. The reaction mixture was refluxed for 10 h in presence of 2% NaOH solution. The resulting mixtures were concentrated and poured onto ice. The completion of reaction was monitored by TLC. The solid thus obtained were filtered, washed and recrystallized with appropriate solvents to furnish compounds 5a-5h.

2-spiro-[5-(5-(2-hydroxyphenyl)amino-3-(5-(2-hydroxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5a: Yield 72% (Ethanol); m.p. 250 °C. IR (KBr, max in cm⁻¹): 3435 (OH), 3339 (NH sym.), 2995 (Aromatic C-H str.), 2220 (-CN str.), 1714 (C=O str.), 1060 (C-O-C); ¹H-NMR (DMSOd₆) in ppm: 3.80 (d, 4H, 2 x CH₂ of isoxazoline ring), 5.87 (d, 2H, 2 x CH of isoxazoline ring), 6.76-7.79 (m, 14H, Ar-H), 8.88 (s, 1H, NH), 11.25 (s, 2H, 2 x OH). Anal. calcd. for C₂₈H₂₃N₅O₆: C, 63.99; H, 4.41; N, 13.33: Found : C, 63.96; H, 4.43; N, 13.36%

2-spiro-[5-(5-(2-methoxyphenyl)amino-3-(5-(2-methoxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5b: Yield 71% (Acetone); m.p. 261 °C. IR (KBr, max in cm⁻¹): 3420 (NH sym.), 2998 (Aromatic C-H str.), 2225 (-CN str.), 1715 (C=O str.), 1294 (N-N), 1065 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 3.83 (d, 4H, 2 x CH₂ of isoxazoline ring), 5.85 (d, 2H, 2 x CH of isoxazoline ring), 6.97-7.87 (m, 14H, Ar-H), 8.85 (s, 1H, NH), 3.50 (s, 6H, 2 x OCH₃). Anal. calcd. for C₃₀H₂₇N₅O₆: C, 65.09; H, 4.92; N, 12.65: Found : C, 65.04; H, 4.95; N, 12.63%

2-spiro-[5-(5-(4-hydroxy-3-methoxyphenyl)amino-3-(5-(4-hydroxy-3-methoxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5c: Yield 68% (Methanol); m.p. 274 °C. IR (KBr, max in cm⁻¹): 3444 (OH), 3420 (NH sym.), 3000 (Aromatic C-H str.), 2235 (-CN str.), 1730 (C=O str.), 1292 (N-N), 1060 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 3.87 (d, 4H, 2 x CH₂ of isoxazoline ring), 5.86 (d, 2H, 2 x CH of isoxazoline ring), 6.74-7.79 (m, 14H, Ar-H), 8.85 (s, 1H, NH), 3.50 (s, 6H, 2 x OCH₃). Anal. calcd. for C₃₀H₂₇N₅O₈: C, 61.53; H, 4.65; N, 11.96: Found : C, 61.52; H, 4.65; N, 11.94%

2-spiro-[5-(5-(4-NN'-dimethylaminophenyl)amino-3-(5-(4-4-NN'-dimethylaminophenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5d: Yield 64% (ethanol); m.p. 264 °C. IR (KBr, max in cm⁻¹): 3444 (OH), 3420 (NH sym.), 3000 (Aromatic C-H str.), 2235 (-CN str.), 1730 (C=O str.), 1294 (N-N), 1071 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 1.32 (s, 12H, 2 x N(CH₃)₂), 3.86 (d, 4H, 2 x CH₂ of isoxazoline ring), 5.82 (d, 2H, 2 x CH of isoxazoline ring), 6.75-7.86 (m, 14H, Ar-H), 8.73 (s, 1H, NH). Anal. calcd. for C₃₂H₃₃N₇O₄: C, 66.31; H, 5.45; N, 16.96%

7-Methoxy-2-spiro-[5-(5-(2-hydroxyphenyl)amino-3-(5-(5-(2-hydroxyphenyl)isoxazolin-3-yl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5e: Yield 68% (petroleum ether); m.p. 271 °C. IR (KBr, max in cm⁻¹): 3454 (OH), 3351 (NH sym.),
Yield 73% (ethanol); m.p. 280°C. IR (KBr, max in cm⁻¹): 3337 (NH sym.), 2220 (-CN str.), 1690 (C=O str.), 1290 (N-N), 1044 (C-O-C); ¹H-NMR (CDCl₃) in ppm: 7.23-7.93 (m, 14H, Ar-H), 8.80 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH₃). Anal. calcd. for C₃₁H₂₉N₅O₇: C, 60.48; H, 4.75; N, 11.38; Found : C, 60.44; H, 4.76; N, 11.34%

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