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To link to this article: http://dx.doi.org/10.1080/00397911.2016.1242748
Novel coumarin isoxazoline derivatives: Synthesis and study of antibacterial activities

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ABSTRACT
A highly efficient and mild protocol for the syntheses of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates and ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates in good yields via [3 + 2] cycloaddition of in situ–generated nitrile oxides from 7-benzyloxy-4-methyl-coumarin hydroxymoylchlorides and 7-benzyloxy-3-chloro-4-methyl-coumarin hydroxymoylchlorides respectively with ethyl-3-aryl prop-2-enoate has been developed. The new compounds are screened for antibacterial activity.

GRAPHICAL ABSTRACT

ARTICLE HISTORY
Received 16 July 2016

KEYWORDS
Antibacterial activity; coumarin isoxazoline; [3 + 2] cycloaddition; 1,3-dipolar cycloaddition; hydroximoyl chloride; nitrile oxide

Introduction

Coumarins (2H-1-benzopyran-2-ones) are important plant-derived metabolites, which exhibit interesting biological and pharmacological activities. Coumarin moiety is used as an important synthetic intermediate and plays a vital role in the synthesis of numerous heterocyclic compounds. Most importantly it is well documented that a large number of pharmaceutical drug products like Novobuocin (antibiotic), Ensaculin (antidementia), and Warfarin (antithrombotic) are marketed widely in the USA, Canada, and the rest of the world and contain 7-hydroxy-4-methyl-2-coumarin as an important structural element. Coumarins containing heterocyclic moieties have significant medicinal value because of their potential pharmacological activities such as antibacterial, antifungal, and antituberculosis activities.

According to the prior literature, compounds containing 2-isoxazoline rings showed enormous biological activities such as antinociceptive, anticonvulsant, antipsychotic,
antistress, and analgesic effects. Moreover, isoxazoline derivatives have played important roles as valuable intermediates in most of the synthetic heterocyclic pharmacological drug products. The traditional synthesis of isoxazolines involves the base-catalyzed cyclocondensation of hydroxyl amine hydrochlorides and chalcones. Kour et al. produce isoxazoline coumarin derivatives using reaction of various substituted chalcone derivatives with hydroxyl amines (free base) in the presence of refluxing methanolic NaOH solution. Desai and coworkers reported the synthesis of isoxazoline coumarin derivatives by cyclocondensation of hydroxyl amine hydrochlorides and substituted chalcones in the presence of refluxing methanolic NaOH solution for 7.5 h. Recently Zghab and coworkers produced coumarin isoxazoline derivatives by cycloaddition of nitrile oxides with suitable substituted ethyl cinnamates in the presence of triethylamine and refluxing toluene. All these synthesis methods are long and require relatively harsh reaction conditions to make coumarin isoxazoline derivatives.

1,3-Dipolar cycloaddition was the most effective method for the synthesis of five-membered heterocycles, which are difficult to prepare by other means. The most widely used method for the synthesis of isoxazolines is the 1,3-dipolar cycloaddition ([3 + 2] cycloaddition) of nitrile oxides to activated dipolarophiles. Aryl nitrile oxides are highly reactive and are predominantly generated in situ by either dehydration of primary nitroalkane derivatives, Mukiyama reaction, or halogenation of aldoxime followed by an in situ dehydrohalogenation using a base. The main advantage of the 1,3-dipolar cycloaddition of aryl nitrile oxides to activated double bond is high regioselectivity with good yields. On the basis of this prior evidence, we set out to prepare a new series of biologically active coumarin appended with isoxazoline pharmacophores 11a–d and 12a–d via [3 + 2] cycloaddition.

As a part of our research for coumarin isoxazoline derivatives, we proposed to investigate the behavior of coumarin-derived aryl nitrile oxides 8 and 9, which are generated in situ from hydroximoylchlorides (aldehyde chlorooximes) 6 and 7 under a basic medium, toward different substituted cinnamates (dipolarophiles) 10. This led to new series of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates 11a–d and ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates 12a–d in good yields. The present study describes the synthesis, characterization, and antibacterial activities of novel coumarin isoxazoline derivatives.

Results and discussion

The required key intermediates for this study, 7-benzyloxy-4-methyl-coumarin aldehyde chlorooximes 6a–d and 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chlorooximes 7a–d, were prepared as shown in Scheme 1. The first step of the synthesis involves the preparation of 7-hydroxy-4-methyl coumarins 2a–d via Pechmann condensation of commercially available resorcinols 1a–d and ethyl acetoacetate in the presence of H2SO4. Further treatment of 7-hydroxy coumarin derivatives 2a–d with hexamine in acetic acid make them undergo Duff formylation to give 8-formyl-7-hydroxy-4-methyl-coumarins 3a–d, which were protected as benzylxy derivatives 4a–d with benzyl bromide in the presence of K2CO3 in dimethylformamide (DMF). Compounds 4a–d treated with hydroxylamine hydrochloride in the presence of methanol and sodium acetate were
converted into oximes 5a–d. Finally, the key intermediates, coumarin-derived hydroximoyl chlorides 6a–d and 7a–d, were synthesized by the chlorination of oximes 5a–d with equimolar quantities of N-chlorosuccinimide in DMF at 20–25 °C. The obtained syrupy mass on purification by column chromatography gave 7-benzyloxy-4-methyl-coumarin aldehyde chloroximes 6a–d and 3-chlorinated products 7a–d as off-white solids. The absence of oxime proton at δ 11.23 in 1H NMR indicates the formation of hydroxymoylchloride derivatives.

Based on our experimental results and in conjunction with known biological properties for heterocycles as an integral part of the coumarin skeleton, we chose useful and stable coumarin-derived hydroximoyl chlorides to synthesize novel coumarin-appended isoxazoline derivatives as shown in Scheme 2. Treatment of coumarin-derived hydroximoyl chlorides 6a–d and 7a–d with triethylamine in dichloromethane at room temperature causes them to undergo dehydrohalogenation and results in situ generation of coumarin aryl nitrile oxides 8a–d and 9a–d, which reacted instantaneously with different ethyl cinnamates 10 (dipolarophiles) via [3 + 2] cycloaddition, yielding the corresponding coumarin isoxazoline derivatives 11a–d and 12a–d. All these newly synthesized compounds were purified by column chromatography and characterized by mass, 1H NMR, and 13C NMR.

To demonstrate the structure elucidation of the first series of coumarin isoxazoline derivatives 11a–d, we selected compound 11a, which was obtained by the reaction of equimolar quantities of 7-benzyloxy-4-methyl-coumarin hydroximoyl chloride 6a and ethyl-3-phenylprop-2-enoate 10 in the presence of triethylamine in dichloromethane at room temperature for 24 h. Its positive quasimolecular ion peak was observed at m/z 483.9 (M+H), compatible with the molecular formulae C29H25NO6. In 1H NMR of 11a the newly formed isoxazoline ring H-4″ appeared at δ 4.68 (d, J = 8.8 Hz) and H-5″ at δ 6.07 (d, J = 8.8 Hz). In 13C NMR of 11a isoxazoline ring carbons appeared at δ 60.5 (C-4″), 88.2 (C-5″), and 158.7 (C-3″).
To demonstrate the structure elucidation of the second series of coumarin isoxazoline derivatives 12a–d, we selected the compound 12a, which was obtained by the reaction of equimolar quantities of 7-benzyloxy-3-chloro-4-methyl-coumarin hydroximoyl chloride 7a and ethyl-3-phenylprop-2-enoate 10. The compound 12a showed its quasimolecular ion peaks at \( m/z \) 518.1 (\( M+H \)) and \( m/z \) 520.3 (\( M+H+2 \)), with 3:1 isotopic peak intensities ratio in mass spectrum confirmed the molecular formulae \( \text{C}_{29}\text{H}_{24}\text{ClNO}_6 \) with one chlorine atom. The newly formed isoxazoline ring proton H-4″ appeared at \( \delta \) 4.64 (d, \( J = 8.6 \) Hz) and H-5″ at \( \delta \) 6.09 (d, \( J = 8.6 \) Hz). In \( ^{13}\text{C} \) NMR of 12a isoxazoline ring carbons appeared at \( \delta \) 62.3 (C-4″), 88.7 (C-5″), and 159.7 (C-3″).

**Antibacterial activity**

It is well known that coumarin isoxazoline derivatives possess antibacterial activity. Furthermore, halogen substituents were introduced into the basic structure, anticipating an improved biological activity, because their incorporation proved to influence the biological activity in various heterocycles as well as coumarins. Electron-withdrawing groups like halogens will increase bactericidal potential as they alter the nature of the compound in such a way as to promote binding to the target(s). According to Prasad et al., designing the compounds bearing electron-withdrawing substituents (with high...
degree of binding linearity) results in high molecular weights to exhibit an improved antibacterial activity.\[22\] Similarly, the significant inhibition shown by substituted isoxazoles was attributed to substituents such as hydroxymethyl/hydroxy/methoxy/ethyl ester groups.\[23\] Shah and Desai also reported that the presence of methoxy-, chloro-, and fluoro groups enhanced the antibacterial activity in isoxazoline derivatives.\[24\]

All these newly synthesized compounds 11a–d and 12a–d were evaluated for in vitro antimicrobial activity against Gram-positive bacteria (Staphylococcus aureus MTCC 96) and Gram-negative bacteria (Escherichia coli MTCC 40) by the agar disc diffusion method.\[25\] The antibacterial screening data showed that most of the compounds 11a–d and 12a–d showed moderate to excellent activities against the used microorganisms (Table- 2) compared to the reference drug (chloramphenicol). These results suggested that the introduction of halogen substituent increased the hydrophobicity of the synthesized compounds and led to the increase of the antibacterial activity.\[26\] In the present study, it is observed that good activity was shown by the prepared derivatives against the studied Gram-positive bacteria and very poor activity against Gram-negative bacteria. The outer cell layers of Gram-positive and Gram-negative bacteria may explain the decent antibacterial activities of these prepared derivatives.\[27\] The permeability of drug constituents in Gram-positive bacteria is due to an ineffective and penetrable outer barrier made of a peptidoglycan layer. On the other hand, outer cell wall of Gram-negative bacteria consists of multiple impermeable peptidoglycan and phospholipidic layers.\[28\] Among the compounds screened, 12c showed high activity. The observed antibacterial activity profile suggested that the presence of halogen functional group bromine had enhanced the activity.

### Table 1. Yields of coumarin isoxazoline derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R1</th>
<th>Product</th>
<th>Yield(^{a}(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>11a</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>Cl</td>
<td>CF3</td>
<td>11b</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>CF3</td>
<td>11c</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>CH(_3)</td>
<td>3,5-difluoro</td>
<td>11d</td>
<td>69</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>H</td>
<td>12a</td>
<td>75</td>
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<tr>
<td>7</td>
<td>Br</td>
<td>CF3</td>
<td>12c</td>
<td>69</td>
</tr>
<tr>
<td>8</td>
<td>CH(_3)</td>
<td>3,5-difluoro</td>
<td>12d</td>
<td>72</td>
</tr>
</tbody>
</table>

\(^a\)Yields are after column purification.

### Table 2. Antibacterial activity data of the synthesized coumarin isoxazoline derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition (mm)(^b)</th>
<th>Escherichia coli (MTCC 40) (Gram-negative) (Conc. (\mu g/ml))</th>
<th>Staphylococcus aureus (MTCC 96) (Gram-positive) (Conc. (\mu g/ml))</th>
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<tr>
<td></td>
<td>200</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>11a</td>
<td>15</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>11b</td>
<td>18</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>11c</td>
<td>27</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>11d</td>
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<td>12</td>
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</tr>
<tr>
<td>12a</td>
<td>22</td>
<td>11</td>
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</tr>
<tr>
<td>12b</td>
<td>11</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>12c</td>
<td>28</td>
<td>24</td>
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</tr>
<tr>
<td>12d</td>
<td>22</td>
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<td>16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>31</td>
<td>30</td>
<td>21</td>
</tr>
</tbody>
</table>

\(^b\)Indicates average of triplicate.
Conclusion

In conclusion, we have successfully achieved two important aspects in this work. One is a highly practical method for the synthesis of novel coumarin-appended isoxazoline derivatives 11a–d and 12a–d in a good yield via [3 + 2] cycloaddition (1,3-dipolar cycloaddition) under mild reaction conditions. The second one is the antibacterial activities of the synthesized compounds. Interestingly all these new compounds are active and show moderate to good antibacterial activity against Gram-positive bacteria and Gram-negative bacteria. The observed antibacterial activity profile suggested that the presence of bromine had enhanced the activity.

Experimental

Melting points were determined in open glass capillaries on a Fisher–Johns melting-point apparatus and are uncorrected. NMR (1H 400 MHz; 13C 125 MHz) were recorded at room temperature in CDCl3 as solvent and tetramethylsilane (TMS) as an internal standard (δ = 0 ppm), and the values were reported in the following order: chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of quartet), coupling constants (J in Hz), and integration. All the reactions were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized under UV light at 254 nm.

Typical experimental procedure for the synthesis of hydroximoyl chlorides (6a–D) and (7a–D)

N-Chlorosuccinimide (4.1 g, 31.0 mmol) was added to a stirred solution of 7-benzyloxy-4-methyl-coumarin aldehyde oxime 5a (8.0 g, 25.8 mmol) in dimethylformamide (DMF, 200.0 mL) at 0 °C, raised to rt over 2 h, quenched with ice cold water (600.0 mL), and extracted with ethyl acetate (3 × 200.0 mL). The combined organic layer was washed with saturated NaCl solution (200.0 mL), dried over Na2SO4, concentrated, and purified by column chromatography to give 6a, 5.6 g (63%). 1H NMR spectrum (400 MHz, CDCl3), δ, ppm (J, Hz): 2.41 (3H, s, 4-CH3); 5.37 (s, 2H, OCH2C6H5); 6.13 (s, 1H, H-3); 7.24–7.35 (5H, m, OCH2C6H5); 7.41 (1H, d, J = 8.8 Hz, H-6); 7.8 (1H, d, J = 8.8 Hz, H-6); 12.4 (1H, s, N-OH) and 7a, 2.6 g (27%); 1H NMR spectrum (400 MHz, CDCl3), δ, ppm (J, Hz): 2.45 (3H, s, 4-CH3); 5.3 (s, 2H, OCH2C6H5); 7.34–7.46 (5H, m, OCH2C6H5); 7.8 (1H, d, J = 8.8 Hz, H-6); 7.9 (1H, d, J = 8.8 Hz, H-6); 12.4 (1H, s, N-OH).

Following the same procedure as depicted for 6a and 7a, the other hydroximoyl chlorides 6b–d and 7b–d were prepared from the corresponding aldehyde oximes. Hydroximoyl chlorides are highly unstable and are readily prepared whenever required.

Typical experimental procedure for the synthesis of coumarin isoxazoline compounds (11a–D)

The corresponding ethyl-3-phenyl-prop-2-enoate 10 (0.29 g, 1.66 mmol) dissolved in dichloromethane (DCM, 12.5 mL) was added to a stirred solution of hydroximoyl chloride 6a (0.5 g, 1.45 mmol) in DCM (12.5 mL) and Et3N (0.19 g, 1.89 mmol), and the reaction
mixture was stirred for 24 h at room temperature. Water (30.0 mL) was added and extracted in DCM (2 × 30.0 mL). The combined organic layer was washed with saturated NaCl solution (60.0 mL), dried over Na₂SO₄, and concentrated to give the crude isoxazoline 11a, which was purified by column chromatography using silica gel in ethyl acetate/petroleum ether (6:4), 0.46 g, 65.7% yield.

Following the same procedure as illustrated for 11a, the other coumarin isoxazoline derivatives 11b–d were prepared from the corresponding hydroximoyl chlorides. The physical, spectral, and analytical data for these compounds are mentioned as follows.

**Ethyl-3-[7-(benzyloxy)-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (11a)**

Off-white solid; yield 0.46 g (66%); mp 130–132 °C; ¹H NMR spectrum (400 MHz, CDCl₃), δ, ppm (J, Hz): 0.95 (3H, t, J = 7.2, OCH₂CH₃); 2.42 (3H, s, 4-CH₃); 3.95 (2H, q, J = 7.2, OCH₂CH₃); 4.68 (1H, d, J = 8.8 Hz, H-4″); 5.21 (2H, s, -OCH₂C₆H₅); 6.07 (1H, d, J = 8.8 Hz, H-5″); 6.29 (s, 1H, H-3); 6.91 (1H, d, J = 9.2 Hz, H-6); 7.23–7.38 (5H, m, OCH₂C₆H₅); 7.51–7.62 (5H, m, Ar); 7.72 (1H, d, J = 9.2 Hz, H-5); ¹³C NMR spectrum (125 MHz, CDCl₃), δ, ppm: 13.7 (OCH₂CH₃); 18.7 (CH₃); 60.5 (C-4″); 63.1 (OCH₂CH₃); 70.1 (OCH₂C₆H₅); 88.2 (C-5″); 103.9 (C-6); 104.2 (C-3); 112.6 (C-4a); 113.9 (C-8); 127.1 (C-2′,6′,4′,2″,6″,4″ Ar); 129.5 (C-3′,5′,3″,5″ Ar); 142.1 (C-5); 150.3 (C-1″, 1); 153.5 (C-8a); 158.7 (C-3″, 4); 166.9 (C-2,7); 167.8 (COOEt); DIPMS: m/z = 483.9 (M+H).

Elemental analysis: found (%): C, 71.92; H, 5.18; N, 2.81. C₂₉H₂₅NO₆ calculated (%): C, 72.04; H, 5.21; N, 2.90.

**Typical experimental procedure for the synthesis of 3-chlorinated coumarin isoxazoline compounds (12a–D)**

The corresponding ethyl-3-phenyl-prop-2-enoate 10 (0.26 g, 1.51 mmol) dissolved in DCM (10.0 mL) was added to a stirred solution of hydroximoyl chloride 7a (0.5 g, 1.32 mmol) in DCM (10.0 mL) and triethylamine (0.17 g, 1.71 mmol) and reaction mixture was stirred for 24 h at room temperature. Water (50.0 mL) was added and extracted with DCM (2 × 50.0 mL). The combined organic layer was washed with saturated NaCl solution (60.0 mL), dried over Na₂SO₄, and concentrated to give the crude isoxazoline, which was purified by column chromatography using silica gel in ethyl acetate/petroleum ether (8:2): 12a, 0.51 g, 74.5% yield.

Following the same procedure as illustrated for 12a, coumarin isoxazoline derivatives 12b–d were prepared from the corresponding hydroximoyl chlorides. The physical, spectral, and analytical data for these compounds are mentioned as follows.

**Ethyl-3-[7-(benzyloxy)-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (12a)**

Off-white solid; yield 0.51 g (75%); mp 118–120 °C; ¹H NMR spectrum (400 MHz, CDCl₃), δ, ppm (J, Hz): 1.03 (3H, t, J = 7.3, OCH₂CH₃); 2.45 (3H, s, 4-CH₃); 3.97 (2H, q, J = 7.3, OCH₂CH₃); 4.64 (1H, d, J = 8.6 Hz, H-4″); 5.24 (2H, s, OCH₂C₆H₅); 6.09 (1H, d,
\[ J = 8.6 \text{ Hz, H-5}^\prime \]; 6.38 (1H, d, J = 9.1 Hz, H-6); 7.28–7.37 (5H, m, OCH\_\_2\_C\_6\_H\_5); 7.51–7.59 (5H, m, Ar); 7.81 (1H, d, J = 9.1 Hz, H-5); \^1^3\text{C} \text{ NMR spectrum (125 MHz, CDCl}_3, \delta, \text{ppm:} 

14.1 (OCH\_2\_CH\_3); 18.9 (CH\_3); 62.3 (C-4'); 64.7 (OCH\_2\_CH\_3); 72.3 (OCH\_2\_C\_6\_H\_5); 88.7 (C-5'); 105.3 (C-6); 113.7 (C-4a); 115.9 (C-8); 119.3 (C-3); 122.5 (C-2',6',4'',6'',4'' Ar); 123.5 (C-3',5',3'',5'' Ar); 142.6 (C-5); 151.8 (C-8a); 154.2 (C-3'''); 163.8 (C-7); 166.9 (COOEt); DIPMS: \text{m/z} = 518.1 (M+H), 520.3 (M+H+2). 

Elemental analysis: Found (%): C, 67.02; H, 4.67; N, 2.69. C\_29\_H\_24\_Cl\_NO\_6: Calculated (%): C, 67.25; H, 4.67; N, 2.70.

### Screening of antibacterial activity

All these newly synthesized compounds, 11a–d and 12a–d, were evaluated for in vitro antimicrobial activity against Gram-positive bacteria (\textit{Staphylococcus aureus} MTCC 96) and Gram-negative bacteria (\textit{Escherichia coli} MTCC 40) by the agar disc diffusion method. All the derivatives at the concentrations of 50 µg/ml, 100 µg/ml, and 200 µg/ml were tested against Gram-positive bacteria (\textit{Staphylococcus aureus} MTCC 96) and Gram-negative bacteria (\textit{Escherichia coli} MTCC 40). The molten nutrient agar was inoculated with 100 µl of the inoculum (1 × 10^8 cfu/ml) and poured into the Petri plate; the disc (5 mm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry, and introduced on the upper layer of the seeded agar plate. The plates were incubated at about 37 °C and microbial progress was determined by measuring the diameter of zone of inhibition after 24 h. Pure solvents were used as control and inhibitory zones were nearly negligible compared to the inhibition zones of the samples. To clarify any solvent effect on the biological screening, separate studies were carried out using dimethyl sulfoxide (DMSO) as a control and it showed no activity against any bacterial strains. Chloramphenicol was used as standard drug for the purpose of comparison of antibacterial activities of the derivatives. The antibacterial activities were carried out in triplicate and average values were compiled.

### References


