SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF 2-IMINO-3-(4-METHOXYPHENYL) OXAZOLIDIN-4-ONE

Tesfahun Bufebo*, Aman Dekebo, Shilashi Badasa² and Hanna Simion

¹Department of Chemistry, Adigrat University, Ethiopia.
²Department of Chemistry, Adama Science and Technology University, P.O. Box 1888, Adama.

ABSTRACT

2-chloro-N-(4-methoxyphenyl)acetamide synthesized by the reaction of 4-methoxybenzenamine with chloroacetylchloride in benzene were cyclocondensed with potassium cyanate in methanol to synthesize 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one and Structure of the synthesized compounds was elucidated by using different spectroscopic techniques such as IR, ¹H NMR, ¹³C NMR and elemental analyses. The purity of the intermediate and the product was checked by TLC plates, the reaction mechanism was given based on known site of functional groups. 2-chloro-N-(4-methoxyphenyl) acetamide was assayed in vitro by paper disc diffusion method for their antibacterial activities against Staphylococcus aureus bacteria, E.coli bacteria, the compound exhibit promising activity even better than standard drugs against tested microorganisms.

KEY WORDS: Iminooxazolidinones, antibacterial, oxazolidinones, spectroscopic and elemental analyses.

INTRODUCTION

The oxazolidinones are a class of antimicrobial agents which have a unique structure and good activity against gram-positive pathogenic bacteria. Oxazolidinones are characterized by presence of 2-oxazolidine moiety in their structure. Oxazolidinones represent a new class of synthetic antibacterial agents active against multiple-resistant gram-positive pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant...
streptococci and vancomycin-resistant enterococci\textsuperscript{[11]}. Oxazolidinones\textsuperscript{[2]} are heterocyclic compounds which perform an important role in the synthesis of several organic molecules including amino acids\textsuperscript{[3]}, amino alcohols\textsuperscript{[4]}, amides\textsuperscript{[5]}, and peptides\textsuperscript{[6]} and polyfunctional compounds\textsuperscript{[7]}. Growing multi-drug resistance by several bacteria, a serious threat in treatment of bacterial infection, warrants urgent search of new antibiotics. This led the development of a new class of oxazolidinone heterocyclics in recent past. Several oxazolidinones, which are a new class of synthetic antibacterial agents, display biological activity against multidrug-resistant Gram-positive organisms\textsuperscript{[8]}. Antibiotics have been prescribed, owing to their effectiveness, on various infectious disorders. However, the appearance of multi-drug-resistant gram-positive bacteria, methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and vancomycin-resistant Enterococci (VRE) in particular caused a serious pain in the neck. Moreover, the emergence of vancomycin-resistant Enterococci (VRE) can be anticipated in the foreseeable future \textsuperscript{[9]}. For the treatment of this Pharmacia is the first compound commercialized worldwide from the oxazolidinone class of antibacterial property to treat multi-drug resistant Gram-positive infections\textsuperscript{[10]}.  

\textbf{MATERIALS AND METHODS}  
4-methoxybenzamine, chloroacetylchloride, benzene, DMSO, CDCl\textsubscript{3}, methanol and KCNO were used in synthetic work. Digital melting point apparatus, silica gel G coated TLC Plates, FTIR-12 spectrophotometer in KBr disc. Brukner Avance spectrometer operating at 400 MHz was used to record \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra in CDCl\textsubscript{3}/DMSO solvent using TMS as an internal standard. Laboratory work such as synthesis of compounds, melting point determination and TLC analysis were done in Haramaya University research laboratory, Ethiopia, NMR analyses were done at Addis Ababa University, Ethiopia, and elemental analysis and FTIR were done in India.  

\textbf{SYNTHETIC PROCEDURE}  
Title compounds, were synthesized by following the reported procedure \textsuperscript{[11]} in two steps as below.  

\textbf{SYNTHESIS OF 2-CHLORO-N-(4-METHOXYPHENYL) ACETAMIDE.}  
For the synthesis of 2-chloro-N-(4-methoxyphenyl) acetamide 32.86 ml chloroacetyl chloride were added drop wise to the solutions 12.3 g 4-methoxybenzamine in benzene with continuous stirring and precipitates obtained were filtered out and washed with benzene repeatedly. Products were air dried and crystallized from acetone to obtain pure compounds.
SYNTHESIS OF 2-IMINO-3-(4-METHOXYPHENYL) OXAZOLIDIN-4-ONE

For the synthesis of 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one (0.025 mole) of 2-chloro-N-(4-methoxyphenyl)acetamide was mixed with (0.15 mole) KCNO in dry methanol and reaction mixtures were refluxed for 3h and filtered to remove KCl. Filtrates were concentrated and residues were washed with water repeatedly to ensure complete removal of KCl. After air drying the products were then recrystallized in ethanol to obtain shining needle type crystals.

Antimicrobial Activities

The synthesized compound was being tested in vitro for their antibacterial activities by using the paper disc diffusion technique[13]. Antibacterial activities of synthesized compounds were tested against a few important bacteria like *Escherichia coli* and *Staphylococcus aureus* using nutrient agar medium. Known antibiotics such as Ampicilin, was used as standard drug as reference in bactericidal.

RESULT AND DISCUSSION

Physico-chemical data of synthesized -2-chloro-N-(4-methoxyphenyl) acetamide and 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one are noted in table 1. Theoretical (calculated) elemental analyses are in fair agreement with experimental values of the products.
Table 1. Molecular formula, colour, melting point, percent yield and analyses of the synthesized compounds

<table>
<thead>
<tr>
<th>S.N</th>
<th>Molecular formula</th>
<th>Colour</th>
<th>Melting point</th>
<th>Yield %</th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₉H₁₀NO₃Cl</td>
<td>Off white</td>
<td>108-110</td>
<td>56</td>
<td>54.2</td>
<td>53.7</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>C₁₀H₁₀N₂O₃</td>
<td>Gray</td>
<td>103-105</td>
<td>63</td>
<td>58.3</td>
<td>58.5</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Characterization of Synthesized Compounds

Reaction of 4-methoxybenzamine with chloroacetylchloride yielded 2-chloro-N-(4-methoxyphenyl) acetamide, with release of HCl and a new C-NH bond is formed. The IR of 2-chloro-N-(4-methoxyphenyl)acetamide displayed one, two or three stretching vibrations in 1349-1312 cm⁻¹ region of C-N group whereas ν N-H displays only one band at 3404-3402 cm⁻¹ amide bond ν C=O display only one band at 1780 cm⁻¹. The presence of these bands characteristic of C-N, N-H C=O group in IR spectra clearly indicates formation of amide bond. Benzene ring bands corresponding to ν C=C and ν C-H vibrations are observed in 1435 cm⁻¹ and 3050 cm⁻¹ regions, respectively. The presence of saturated CH₃ in IR spectra was clearly indicated in C-H stretching at 2850 cm⁻¹.

Cyclocondensation of 2-chloro-N-(4-methoxyphenyl)acetamide with potassium cyanate lead to the formation of 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one containing C=O, C=N, C-N, C-H (saturated aliphatic) N-H and C-O-C group and elimination of KCl. IR spectra of 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one displayed bands corresponding to ν C=O, ν C=N, ν C-H, ν N-H and ν C-O-C vibrations in 1665-1684 cm⁻¹, 1684 cm⁻¹, 2741-2856 cm⁻¹, 3404-3402 cm⁻¹ and 636-725 cm⁻¹ regions, respectively. The absence of strong absorption band between 2280-2100 cm⁻¹ also indicate the formation of 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one because the functional group nitrogen carbon triple bond of potassium cyanate is absent in 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one.

Table 2. IR frequencies of characteristic groups of 2-chloro-N-(4-methoxyphenyl) acetamide and 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one in cm⁻¹

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>νC=O</th>
<th>νC=N</th>
<th>νC-O in ring</th>
<th>νC-H Str</th>
<th>νC-O-C</th>
<th>νN-H</th>
<th>Aromatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₉H₁₀NO₃Cl</td>
<td>1780</td>
<td>-</td>
<td>-</td>
<td>2850</td>
<td>-</td>
<td>3420</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2850</td>
<td></td>
<td></td>
<td>142-145</td>
</tr>
<tr>
<td>2</td>
<td>C₁₀H₁₀N₂O₃</td>
<td>1665-1684</td>
<td>1684</td>
<td>1122</td>
<td>2741-1856</td>
<td>636-725</td>
<td>3404-3404</td>
<td>142-145</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1856</td>
<td></td>
<td></td>
<td>2957</td>
</tr>
</tbody>
</table>
With a view to receive further NMR verification of IR spectra inference regarding structure of 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one $^1$H NMR of has been critically analyzed. 3H of OCH$_3$, 4Hs of aromatic, 1H of enol (=C-OH) and 2H of CH$_2$ in ring displaying the characteristic proton signals at chemical shifts $\delta$ 3.5, $\delta$ 6.5-6.75, $\delta$ 9.7 and $\delta$ 3.8 regions indicate the presence of OCH$_3$, aromatic H, enol protons and aliphatic CH$_2$ of 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one respectively. The structural inferences of the products using $^1$H NMR, spectral data provide substantial support to IR results. In $^{13}$C NMR spectra of the synthesized compounds indicate a peak in 160 ppm region due to the carbonyl carbon of the 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one ring. Carbon signals at 156 ppm is due to C=NH, signals at 76 ppm due to CH$_2$ sandwiched between oxygen and carbonyl group in 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one ring, peak in 55 ppm is aliphatic of CH$_3$ group attached with oxygen, signal at 147 ppm is due to quaternary oxygenated aromatic carbon. Signals at 101-138 ppm indicate presence of aromatic methine carbons. Thus $^{13}$C NMR spectra of the compounds provide strong support to IR and $^1$H NMR structural results. IR, $^1$H NMR and $^{13}$C NMR spectral assignments of these compounds were in good agreement with those reported in literature$^{[12]}$.

**Reaction Mechanism of Synthesized Compounds**

![Scheme 3: Reaction mechanism for conversion of 4-methoxybenzenamine to 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one](image)

**Antimicrobial Effects**
The in vitro antimicrobial activity of compounds 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one was performed using the disk diffusion method. Ampicillin was used as standard drugs for bacteria. The compounds 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one was tested for their anti-bacterial activities by disk-diffusion method using nutrient broth medium. Perusal of antibacterial data of 2-imino-3-(4-methoxyphenyl) oxazolidin-4-one shows activity of against both *E. coli* and *S. aureus* bacteria. The compound show high activity against both S.
aureus and E. coli bacteria in 20 µl concentrations, the compound also show medium in both S. aureus and E. coli bacteria in 10 µl concentrations. From this activity it could be easily inferred that bactericidal activity is dependent on position of substituent, concentration and nature compound. The outcome of this study is presented in table-3.

Table 3. Zone of bacterial growth inhibition (mm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>Zone of inhibition (mm)</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 µl 20 µl</td>
<td>10 µl 20 µl</td>
</tr>
<tr>
<td>1</td>
<td>C_{10}H_{10}N_{2}O_{3}</td>
<td>6.5 8.5</td>
<td>9 11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ampiciln</td>
<td>8 10</td>
<td>11 12</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DMSO</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
</tbody>
</table>

Key: all results mean of three replications no zone of inhibition observed

CONCLUSIONS
An efficient cyclization protocol for 2-chloro-N-(4-methoxyphenyl)acetamide with potassium cyanate to novel 2-imino-3-(4-methoxyphenyl)oxazolidin-4-one reported earlier has been followed owing to its simplicity of the various target experimental procedure and good yield. Homogeneity of the synthesized products was tested using thin-layer chromatography. Their IR, $^1$H NMR and $^{13}$C NMR spectra were used to elucidate structures of the synthesized compounds. The importance of such work lies in the possibility that the new synthesized compound might be a more efficacious drug against various bacterial species for which a thorough investigation regarding the structure activity relationship, mechanism of action and toxicity in their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use.

REFERENCE


