



## Synthesis of Some Novel Isoxazolidine Derivatives via 1,3-Dipolar Cycloaddition and Their Biological Evaluation

Volkan YANMAZ<sup>1</sup> , Ali DISLI<sup>1</sup> , Serkan YAVUZ<sup>1</sup> , Hatice OGUTCU<sup>2</sup> , Gulay DILEK<sup>3,\*</sup> 

<sup>1</sup>Gazi University, Department of Chemistry, 06500, Ankara, Turkey

<sup>2</sup>Ahi Evran University, Department of Biology, 40100, Kırşehir, Turkey

<sup>3</sup>Zonguldak Bulent Ecevit University, Department of Basic Pharmaceutical Sciences, 67600, Zonguldak, Turkey

### Article Info

Received: 24/01/2018

Accepted: 05/10/2018

### Keywords

Isoxazolidine,  
Nitrone  
1,3-Dipolar  
cycloaddition reaction  
Antimicrobial activity

### Abstract

A series of novel substituted isoxazolidine derivatives were synthesized by 1,3-dipolar cycloaddition reactions of  $\alpha$ -aryl-*N*-methyl nitrones with diethyl maleate. The structures of the synthesized compounds were characterized using spectroscopic methods. All the synthesized compounds were screened for their antibacterial activities against Gram-positive and Gram-negative bacteria and for their antifungal activities against a yeast strain. The results show that all the synthesized compounds displayed significant activity against *S. epidermidis*, *M. luteus*, *B. cereus*, *B. abortus* and *C. albicans* when compared to standard drugs.

## 1. INTRODUCTION

Inter- and intramolecular cycloaddition reactions are one of the most efficient and fundamental tools for the synthesis of novel cyclic scaffolds [1]. The addition of 1,3-dipoles such as nitrones, nitrile oxides, azomethine ylides, diazocarbonyls and nitronates to an alkene has received considerable attention in asymmetric synthesis [2]. It allows up to three contiguous stereogenic centers to be created in a single step [3].

1,3-Dipolar cycloaddition reaction of nitrones with alkenes is mostly applied for the synthesis of isoxazolidine derivatives [4]. Isoxazolidines, five membered heterocycles containing neighboring nitrogen and oxygen atoms, are valuable synthons for the synthesis of simple and complex molecules [5]. As a result of the labile nature of the N-O bond under mild reducing conditions [6], these derivatives are considered important synthetic intermediates to generate natural products such as alkaloids [7], amino sugars [8], nucleoside analogues [9], penem and carbapenem antibiotics [10], vitamin D analogues [11],  $\beta$ -amino acids and  $\gamma$ -amino alcohols [12]. In addition, the isoxazolinyl and related compounds show great promise as drug candidates [13]. They are known to possess antibacterial, antifungal [14,15], insecticidal [16], antituberculosis [17], antiinflammatory [18], analgesic [19], antithrombotic [20], antitumor and antiviral activities [21]. Considering the interest shown in these compounds, we herein report the synthesis and antimicrobial activities of some novel isoxazolidine derivatives.

\*Corresponding author, e-mail: [gulaydilekcelik@hotmail.com](mailto:gulaydilekcelik@hotmail.com)

## 2. EXPERIMENTAL

All chemicals and solvents used were reagent grade (Merck or Aldrich) and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on 60 F<sub>254</sub> precoated silica gel plates (Merck). Plates were visualized by UV light. Column chromatography was performed using Merck Silica Gel 60 F<sub>254</sub> (particle size: 0.63-0.200 mm; 70-230 mesh ASTM). Melting points were determined with an Electrothermal 9100 melting point apparatus and are uncorrected. IR spectra were recorded on a Mattson 1000 FTIR spectrometer (Mattson Instruments, Baton Rouge, LA) in KBr disks and were reported in cm<sup>-1</sup> units. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR were obtained in CDCl<sub>3</sub> (Merck) and recorded with a Bruker Spectrospin Avance DPX-300 Ultrashield instrument using TMS as an internal reference (chemical shift in δ ppm). Mass spectra measurements were recorded on a Waters ACQUITY ultra performance liquid chromatography combined with Micromass LCT Premier™ XE TOF-MS and electrospray ionization (Waters Corporation, Milferd, MA); Experimental high-resolution mass results are in good agreement with calculated values.

### 2.1. Synthesis of α-Aryl-N-methyl nitrones (2a-2i)

A mixture of aromatic aldehyde (10 mmol), *N*-methylhydroxylamine hydrochloride (1.65 g, 20 mmol), potassium carbonate (3.04 g, 22 mmol) and sodium sulphate (0.71 g, 5 mmol) were added in a mortar and ground rapidly with a pestle at room temperature. The reaction was monitored by thin layer chromatography. Diethyl ether (25 ml) was added to the crude product mixture and filtered. The solvent was evaporated *in vacuo* and the residue was crystallized from petroleum ether [22]. Chemical structures of the compounds **2a-2i** were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra and melting points reported [23-26]. They were in accordance with the reported literature values. Synthesized α-aryl-*N*-methyl nitrones **2a-2i** (in 77-92% yield) were directly used for the next reaction without further purification.

### 2.2. Synthesis of Isoxazolidine Derivatives (3a-3i, 4a-4c, 4e-4f and 4h)

The appropriate α-aryl-*N*-methyl nitrone (3.0 mmol) was refluxed with dimethyl maleate (0.48 g, 3.3 mmol) in toluene (50 ml) for 8 h. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using ethyl acetate-hexane (2:1) as eluent and recrystallized from chloroform-hexane mixtures to give the corresponding isoxazolidine derivatives **3a-3i**, **4a-4c**, **4e-4f** and **4h** [22]. Compounds **4d**, **4g** and **4i** could not be isolated.

#### 2.2.1. (3R,4R,5S)-Dimethyl 3-(4-Fluorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3a)

Yield: 43%, mp. 112-113 °C. IR (KBr): ν 1214 (-N-O), 1733 (-C=O), 2947 (-C-H), 3042 cm<sup>-1</sup> (Ar-H). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 2.72 (s, 3H, -N-CH<sub>3</sub>), 3.66 (s, 3H, -O-CH<sub>3</sub>), 3.69 (dd, 1H, *J* = 9.1 Hz, -C<sub>4</sub>H), 3.79 (s, 3H, -O-CH<sub>3</sub>), 3.95 (d, 1H, *J* = 9.2 Hz, -C<sub>3</sub>H), 4.93 (d, 1H, *J* = 9.1 Hz, -C<sub>5</sub>H), 7.02-7.41 ppm (m, 4H, Ar-H). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): δ 42.8, 52.3, 52.4, 59.6, 74.7, 76.8, 116.1, 116.5, 129.5, 129.6, 131.6, 164.5, 168.9, 169.8 ppm. HR-MS for C<sub>14</sub>H<sub>17</sub>FNO<sub>5</sub><sup>+</sup> ([M+H]<sup>+</sup>) Calcd. 298.1091; Found 298.1086.

#### 2.2.2. (3S,4R,5S)-Dimethyl 3-(4-Fluorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (4a)

Yield: 38%, mp. 135-136 °C. IR (KBr): ν 1207 (-N-O), 1725 (-C=O), 2973 (-C-H), 3030 cm<sup>-1</sup> (Ar-H). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 2.69 (s, 3H, -N-CH<sub>3</sub>), 3.24 (s, 3H, -O-CH<sub>3</sub>), 3.79 (s, 3H, -O-CH<sub>3</sub>), 4.08 (dd, 1H, *J* = 8.7 Hz, -C<sub>4</sub>H), 4.19 (d, 1H, *J* = 8.3 Hz, -C<sub>3</sub>H), 4.94 (d, 1H, *J* = 8.3 Hz, -C<sub>5</sub>H), 6.99-7.37 ppm (m, 4H, Ar-H). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): δ 43.4, 51.8, 52.3, 57.8, 74.5, 75.8, 115.3, 115.6, 129.7, 129.8, 161.1, 164.3, 168.9, 170.3 ppm. HR-MS for C<sub>14</sub>H<sub>17</sub>FNO<sub>5</sub><sup>+</sup> ([M+H]<sup>+</sup>) Calcd. 298.1091; Found 298.1072.

**2.2.3. (3R,4R,5S)-Dimethyl 3-(2-Fluorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3b)**

Yield: 41%, mp. 79-80 °C. IR (KBr):  $\nu$  1203 (-N-O), 1721 (-C=O), 2950 (-C-H), 3010  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.70 (s, 3H, -O-CH<sub>3</sub>), 3.75 (s, 3H, -O-CH<sub>3</sub>), 3.80 (dd, 1H,  $J = 8.6$  Hz, -C<sub>4</sub>H), 4.25 (d, 1H,  $J = 8.5$  Hz, -C<sub>3</sub>H), 4.95 (d, 1H,  $J = 8.8$  Hz, -C<sub>5</sub>H), 7.04-7.54 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  42.9, 52.5, 58.2, 69.1, 76.6, 115.8, 123.0, 124.7, 129.0, 130.3, 159.5, 162.8, 168.6, 170.1 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{FNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 298.1091; Found 298.1083.

**2.2.4. (3S,4R,5S)-Dimethyl 3-(2-Fluorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (4b)**

Yield: 26%, mp. 98-99 °C. IR (KBr):  $\nu$  1203 (-N-O), 1735 and 1758 (-C=O), 2950 (-C-H), 3050  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.75 (s, 3H, -N-CH<sub>3</sub>), 3.20 (s, 3H, -O-CH<sub>3</sub>), 3.80 (s, 3H, -O-CH<sub>3</sub>), 4.20 (dd, 1H,  $J = 8.6$  Hz, -C<sub>4</sub>H), 4.35 (d, 1H,  $J = 8.5$  Hz, -C<sub>3</sub>H), 4.96 (d, 1H,  $J = 8.6$  Hz, -C<sub>5</sub>H), 7.01-7.52 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  51.7, 52.4, 56.2, 75.8, 114.9, 115.1, 124.2, 124.3, 128.4, 129.9, 159.2, 162.4 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{FNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 298.1091; Found 298.1085.

**2.2.5. (3R,4R,5S)-Dimethyl 3-(4-Chlorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3c)**

Yield: 45%, mp. 84-85 °C. IR (KBr):  $\nu$  1233 (-N-O), 1723 (-C=O), 2947 (-C-H), 3042  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.65 (s, 3H, -O-CH<sub>3</sub>), 3.67 (dd, 1H,  $J = 9.1$  Hz, -C<sub>4</sub>H), 3.78 (s, 3H, -O-CH<sub>3</sub>), 3.92 (d, 1H,  $J = 9.2$  Hz, -C<sub>3</sub>H), 4.90 (d, 1H,  $J = 9.1$  Hz, -C<sub>5</sub>H), 7.26-7.33 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  43.0, 52.4, 52.5, 59.8, 74.8, 76.7, 129.1, 129.2, 134.5, 134.8, 168.7, 169.9 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{ClNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 314.0795; Found 314.0792.

**2.2.6. (3S,4R,5S)-Dimethyl 3-(4-Chlorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (4c)**

Yield: 22%, mp. 101-102 °C. IR (KBr):  $\nu$  1207 (-N-O), 1721 (-C=O), 2967 (-C-H), 3020  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.30 (s, 3H, -O-CH<sub>3</sub>), 3.80 (s, 3H, -O-CH<sub>3</sub>), 4.05 (dd, 1H,  $J = 8.7$  Hz, -C<sub>4</sub>H), 4.20 (d, 1H,  $J = 8.4$  Hz, -C<sub>3</sub>H), 4.95 (d, 1H,  $J = 8.4$  Hz, -C<sub>5</sub>H), 7.25-7.39 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  43.5, 51.8, 52.4, 57.8, 74.6, 75.8, 128.7, 129.4, 134.5, 168.8, 168.9 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{ClNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 314.0795; Found 314.0789.

**2.2.7. (3R,4R,5S)-Dimethyl 3-(2-Chlorophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3d)**

Yield: 44%, mp. 161-162 °C. IR (KBr):  $\nu$  1203 (-N-O), 1725 (-C=O), 2950 (-C-H), 3066  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.65 (dd, 1H,  $J = 8.6$  Hz, -C<sub>4</sub>H), 3.68 (s, 3H, -O-CH<sub>3</sub>), 3.79 (s, 3H, -O-CH<sub>3</sub>), 4.52 (d, 1H,  $J = 8.0$  Hz, -C<sub>3</sub>H), 4.90 (d, 1H,  $J = 8.7$  Hz, -C<sub>5</sub>H), 7.21-7.64 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  43.0, 52.4, 52.5, 59.3, 71.7, 76.7, 127.5, 128.7, 129.5, 129.9, 134.2, 134.7, 168.6, 170.5 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{ClNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 314.0795; Found 314.0802.

**2.2.8. (3R,4R,5S)-Dimethyl 3-(4-Bromophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3e)**

Yield: 41%, mp. 94-95 °C. IR (KBr):  $\nu$  1213 (-N-O), 1735 and 1761 (-C=O), 2983 (-C-H), 3026  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.65 (s, 3H, -O-CH<sub>3</sub>), 3.70 (dd, 1H,  $J = 9.0$  Hz, -C<sub>4</sub>H), 3.79 (s, 3H, -O-CH<sub>3</sub>), 3.92 (d, 1H,  $J = 9.2$  Hz, -C<sub>3</sub>H), 4.91 (d, 1H,  $J = 9.1$  Hz, -C<sub>5</sub>H), 7.28-7.50 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  43.0, 52.4, 52.5, 59.8, 74.8, 76.6, 122.7, 129.5, 132.1, 135.4, 168.9, 169.9 ppm. HR-MS for  $\text{C}_{14}\text{H}_{17}\text{BrNO}_5^+$  ( $[\text{M}+\text{H}]^+$ ) Calcd. 358.0290; Found 358.0290.

**2.2.9. (3S,4R,5S)-Dimethyl 3-(4-Bromophenyl)-2-methylisoxazolidine-4,5-dicarboxylate (4e)**

Yield: 28%, mp. 81-82 °C. IR (KBr):  $\nu$  1216 (-N-O), 1731 and 1754 (-C=O), 2987 (-C-H), 3026  $\text{cm}^{-1}$  (Ar-H).  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.70 (s, 3H, -N-CH<sub>3</sub>), 3.32 (s, 3H, -O-CH<sub>3</sub>), 3.78 (s, 3H, -O-CH<sub>3</sub>), 3.91 (dd, 1H,  $J = 8.7$  Hz, -C<sub>4</sub>H), 4.09 (d, 1H,  $J = 8.4$  Hz, -C<sub>3</sub>H), 4.90 (d, 1H,  $J = 8.4$  Hz, -C<sub>5</sub>H), 7.24-7.52 ppm (m, 4H, Ar-H).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  42.7, 52.4, 52.5, 59.4, 75.5, 76.9, 127.9, 129.1, 129.2, 129.5,

129.7, 138.8, 168.9, 170.0 ppm. HR-MS for  $C_{14}H_{17}BrNO_5^+$  ( $[M+H]^+$ ) Calcd. 358.0290; Found 358.0298 [22].

#### 2.2.10. (3R,4R,5S)-Dimethyl 2-Methyl-3-p-tolylisoxazolidine-4,5-dicarboxylate (3f)

Yield: 37%, mp. 133-134 °C. IR (KBr):  $\nu$  1216 (-N-O), 1716 and 1721 (-C=O), 2960 (-C-H), 3033  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.34 (s, 3H, Ar- $CH_3$ ), 2.72 (s, 3H, -N- $CH_3$ ), 3.65 (s, 3H, -O- $CH_3$ ), 3.79 (dd, 1H,  $J = 8.9$  Hz, - $C_4H$ ), 3.80 (s, 3H, -O- $CH_3$ ), 3.93 (d, 1H,  $J = 9.1$  Hz, - $C_3H$ ), 4.95 (d, 1H,  $J = 9.1$  Hz, - $C_5H$ ), 7.16-7.32 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  21.2, 42.8, 52.4, 52.8, 57.3, 75.5, 76.9, 127.9, 129.5, 134.3, 138.3, 168.9, 170.1 ppm. HR-MS for  $C_{15}H_{20}NO_5^+$  ( $[M+H]^+$ ) Calcd. 294.1341; Found 294.1339 [27]

#### 2.2.11. (3S,4R,5S)-Dimethyl 2-Methyl-3-p-tolylisoxazolidine-4,5-dicarboxylate (4f)

Yield: 19%, mp. 141-142 °C. IR (KBr):  $\nu$  1216 (-N-O), 1716 and 1721 (-C=O), 2960 (-C-H), 3033  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.36 (s, 3H, Ar- $CH_3$ ), 2.76 (s, 3H, -N- $CH_3$ ), 3.31 (s, 3H, -O- $CH_3$ ), 3.75 (s, 3H, -O- $CH_3$ ), 3.98 (dd, 1H,  $J = 8.6$  Hz, - $C_4H$ ), 4.06 (d, 1H,  $J = 8.7$  Hz, - $C_3H$ ), 4.90 (d, 1H,  $J = 8.6$  Hz, - $C_5H$ ), 7.11-7.26 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  21.2, 43.7, 51.8, 52.4, 58.2, 75.4, 75.8, 127.7, 127.8, 129.1, 129.2, 130.7, 138.3, 169.2 ppm. HR-MS for  $C_{15}H_{20}NO_5^+$  ( $[M+H]^+$ ) Calcd. 294.1341; Found 294.1333.

#### 2.2.12. (3R,4R,5S)-Dimethyl 2-Methyl-3-o-tolylisoxazolidine-4,5-dicarboxylate (3g)

Yield: 46%, mp. 144-145 °C. IR (KBr):  $\nu$  1200 (-N-O), 1718 (-C=O), 2957 (-C-H), 3033  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.35 (s, 3H, Ar- $CH_3$ ), 2.69 (s, 3H, -N- $CH_3$ ), 3.65 (s, 3H, -O- $CH_3$ ), 3.75 (dd, 1H,  $J = 9.0$  Hz, - $C_4H$ ), 3.80 (s, 3H, -O- $CH_3$ ), 4.30 (d, 1H,  $J = 8.8$  Hz, - $C_3H$ ), 4.97 (d, 1H,  $J = 9.1$  Hz, - $C_5H$ ), 7.14-7.53 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  19.5, 42.6, 52.3, 52.4, 59.3, 71.6, 77.1, 126.6, 127.4, 128.1, 130.8, 133.8, 137.1, 168.8, 170.4 ppm. HR-MS for  $C_{15}H_{20}NO_5^+$  ( $[M+H]^+$ ) Calcd. 294.1341; Found 294.1322.

#### 2.2.13. (3R,4R,5S)-Dimethyl 3-(4-Methoxyphenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3h)

Yield: 38%, mp. 121-122 °C. IR (KBr):  $\nu$  1213 (-N-O), 1731 and 1754 (-C=O), 2960 (-C-H), 3056  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.69 (s, 3H, -N- $CH_3$ ), 3.64 (s, 3H, -O- $CH_3$ ), 3.71 (dd, 1H,  $J = 9.0$  Hz, - $C_4H$ ), 3.80 (s, 3H, -O- $CH_3$ ), 3.83 (s, 3H, Ar-O- $CH_3$ ), 3.87 (d, 1H,  $J = 9.2$  Hz, - $C_3H$ ), 4.91 (d, 1H,  $J = 9.1$  Hz, - $C_5H$ ), 6.89-7.29 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  43.7, 51.8, 52.4, 58.2, 75.4, 76.7, 127.8, 129.2, 130.7, 138.3, 169.2, 170.2 ppm. HR-MS for  $C_{15}H_{20}NO_6^+$  ( $[M+H]^+$ ) Calcd. 310.1291; Found 310.1292.

#### 2.2.14. (3S,4R,5S)-Dimethyl 3-(4-Methoxyphenyl)-2-methylisoxazolidine-4,5-dicarboxylate (4h)

Yield: 23%, mp. 129-130 °C. IR (KBr):  $\nu$  1221 (-N-O), 1754 (-C=O), 2952 (-C-H), 3047  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.70 (s, 3H, -N- $CH_3$ ), 3.28 (s, 3H, -O- $CH_3$ ), 3.78 (s, 3H, -O- $CH_3$ ), 3.84 (s, 3H, Ar-O- $CH_3$ ), 3.95 (dd, 1H,  $J = 8.9$  Hz, - $C_4H$ ), 4.03 (d, 1H,  $J = 8.7$  Hz, - $C_3H$ ), 4.89 (d, 1H,  $J = 8.6$  Hz, - $C_5H$ ), 6.84-7.26 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  43.6, 51.8, 52.3, 55.2, 58.1, 75.1, 75.7, 113.8, 125.7, 129.0, 129.1, 129.2, 159.6, 169.3, 170.7 ppm. HR-MS for  $C_{15}H_{20}NO_6^+$  ( $[M+H]^+$ ) Calcd. 310.1291; Found 310.1286.

#### 2.2.15. (3R,4R,5S)-Dimethyl 3-(2-Methoxyphenyl)-2-methylisoxazolidine-4,5-dicarboxylate (3i)

Yield: 43%, mp. 87-88 °C. IR (KBr):  $\nu$  1214 (-N-O), 1723 and 1771 (-C=O), 2990 (-C-H), 3033  $cm^{-1}$  (Ar-H).  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  2.80 (s, 3H, -N- $CH_3$ ), 3.65 (s, 3H, -O- $CH_3$ ), 3.69 (dd, 1H,  $J = 8.5$  Hz, - $C_4H$ ), 3.80 (s, 3H, -O- $CH_3$ ), 3.82 (s, 3H, Ar-O- $CH_3$ ), 4.34 (d, 1H,  $J = 8.0$  Hz, - $C_3H$ ), 4.90 (d, 1H,  $J = 8.7$  Hz, - $C_5H$ ), 6.87-7.46 ppm (m, 4H, Ar-H).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$  43.2, 52.2, 52.4, 55.4, 58.2, 70.4,

77.2, 110.8, 120.9, 124.8, 127.9, 129.3, 157.6, 168.8, 171.2 ppm. HR-MS for  $C_{15}H_{20}NO_6^+$  ( $[M+H]^+$ ) Calcd. 310.1291; Found 310.1286.

### 2.3. Antimicrobial Activity Studies

The *in vitro* antibacterial activity of isoxazolidine derivatives **3a-3i**, **4a-4c**, **4e-4f** and **4h** was performed against American Type Culture Collection (ATCC) reference bacterial strain and fungi by disc diffusion technique [28,29]. Gram-negative and Gram-positive bacteria were grown in nutrient agar medium and incubated at 37 °C for 24 h. The yeast strain was grown in Sabouraud dextrose agar medium and incubated at 27 °C for 72 hours. Kanamycin, ampicillin, chloramphenicol, amoxicillin, ofloxacin and sulbactam were used as reference antibacterial agents, and nystatin was used as a reference antifungal agent under similar conditions for comparison. Dimethyl sulfoxide (DMSO) was used as a control. All the testings were repeated for three times.

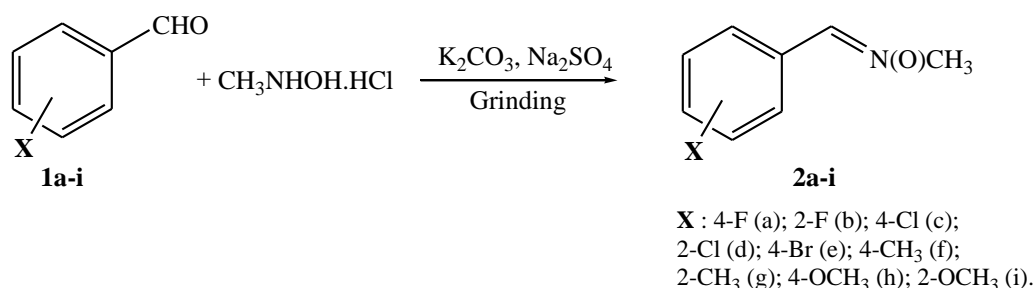
### 2.4. Statistical Analysis

Statistical analyses were performed in SPSS 19.0 package software. Descriptive statistics were given with median, minimum and maximum values. Mann Whitney U test were used for the two group comparisons. Kruskal-Wallis test was used for the three group comparisons. Bonferroni corrected Mann Whitney U test was used for post-hoc comparisons after Kruskal Wallis test. For all statistical comparisons with a p value below 0.05 assumed as statistically significant.

## 3. RESULTS AND DISCUSSION

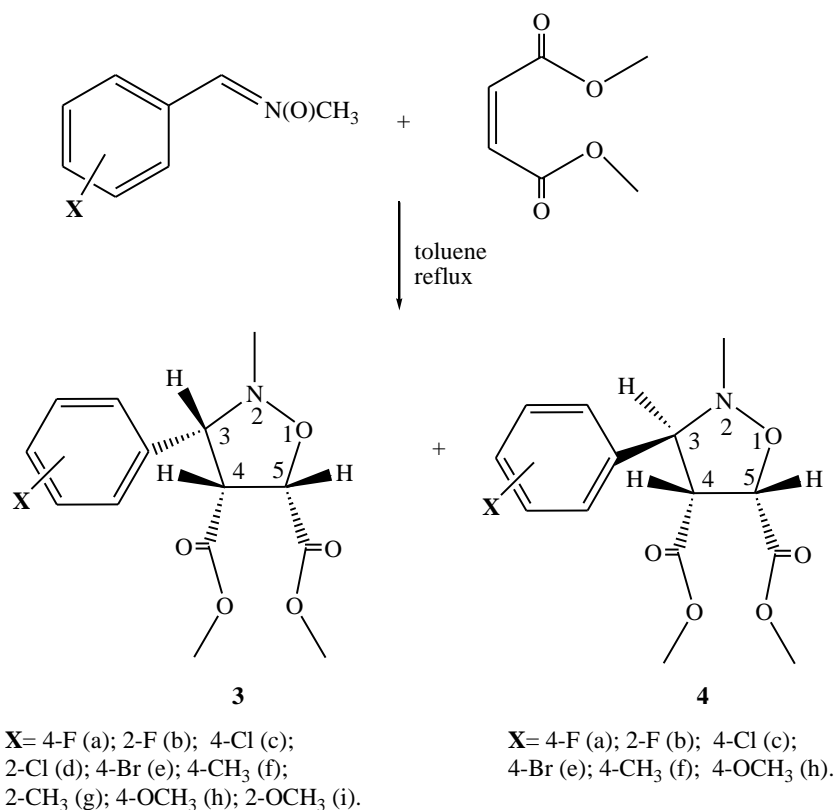
1,3-Dipolar cycloaddition reactions of nitrones with olefinic dipolarophiles are an efficient method for the synthesis of the isoxazolidine ring systems [30]. Nitrones are generally prepared by alkylation of oximes, dehydrogenation of *N,N*-disubstituted hydroxylamines or condensation of *N*-methylhydroxylamine hydrochloride with aromatic aldehydes [31,32]. These methods usually require warming or even refluxing in organic solvents. Besides, water forms during condensation, if not removed it, hydrolyzes nitron and thus diminishes the reaction yields [31]. Solvent-free reaction procedures have received tremendous attention in recent years due to mild reaction conditions, less by-products, easy separation and purification, increase in rate of reaction and high degree of stereoselectivity [33,34].

In the present work, the synthesis of isoxazolidine derivatives were carried out in two steps. In the first step,  $\alpha$ -aryl-*N*-methyl nitrones (**2a-2i**) were synthesized by grinding the substituted benzaldehydes (**1a-1i**) and *N*-methylhydroxylamine hydrochloride in the presence of  $K_2CO_3$ - $Na_2SO_4$  at room temperature under solvent-free conditions (Scheme 1). The overall yields of the reactions were high (77-92 %). Chemical structures of the compounds **2a-2i** were confirmed by their  $^1H$ -NMR,  $^{13}C$ -NMR spectra and melting points reported [23-26].



**Figure 1.** Synthetic pathway for the preparation of *N*-methyl nitrones **2a-2i**

In the second step, the novel isoxazolidine derivatives were prepared by refluxing  $\alpha$ -aryl-*N*-methyl nitrones (**2a-2i**) with dimethyl maleate in toluene (Scheme 2). The *cis*-isomers (**3a-3i**) and *trans*-isomers (**4a-4c**, **4e-4f** and **4h**) of cycloadducts were separated and purified using column chromatography. Compounds **4d**, **4g** and **4i** could not be isolated. The structures of the synthesized *cis*- and *trans*-isomers of isoxazolidine derivatives were identified by spectroscopic methods (FT-IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and HR-MS spectra). Crystal structures of compounds **3f** and **4e** were already confirmed by X-ray diffraction [22,27].



**Figure 2.** Synthetic pathway for the preparation of compounds **3a-3i**, **4a-4c**, **4e-4f** and **4h**

### 3.1. Antimicrobial Activity Results

The *in vitro* antibacterial activities of *cis*-isomers (**3a-3i**) and *trans*-isomers (**4a-4c**, **4e-4f** and **4h**) were performed against Gram-positive bacteria including *Staphylococcus aureus* RSKK 07035, *Listeria monocytogenes* ATCC 19115 (serotype 4b), *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus cereus* (Table 1) and Gram-negative bacteria including *Shigella dysenteriae* NCTC 9363 (type 7), *Escherichia coli* ATCC 1230, *Salmonella typhi* H NCTC 901.8394, *Brucella abortus* RSKK 03026, *Pseudomonas putida* by disc diffusion technique (Table 2) [28,29]. In addition, the *in vitro* antifungal activity of the compounds was performed against *Candida albicans* Y 1200 NIH (Table 2).

Isoxazolidine derivatives exhibit a broad spectrum of biological activities [10,35-42]. They exhibit antibacterial activities in which some compounds are good and some are moderately active when compared with standard drugs tested [10,35-42]. From the studies, it is known that *N*-substituted isoxazolidines show antifungicidal properties [42]. Moreover, 3,5-disubstituted isoxazolidines exhibit antifungal and antimicrobial activities against fungus and microorganisms [41]. The activity of these compounds is similar or even better than standard drugs. Regarding these studies, it may be concluded that presence of -COOR, -COOH, -OH and -Ar groups at C-3 and C-5 positions increases antibacterial activity [40,41]. Likely, these groups on isoxazolidine ring may afford an adequate equilibrium between hydrophobicity and polarity that is crucial for overall potency [41].

In this study, all the synthesized isoxazolidine compounds were mostly effective against bacteria and the yeast strain, *C.albicans*. They were even found to be effective against *S. epidermidis*, *M. luteus*, *B. cereus*,

*B. abortus* and *C. albicans*, while standard drugs kanamycin, ampicillin and chloramphenicol did not display any activity against them. Besides, the compounds **3b**, **3f**, **3g**, **3h**, **4e**, **4f** and **4h** were almost competitive with the standard antifungal drug, nystatin.

**Table 1.** Antimicrobial activities of compounds *cis*-isomers (**3a-3i**) and *trans*-isomers (**4a-4c**, **4e-4f**, **4h**) against Gram-positive bacteria

Inhibition zone diameter (mm)					
Compounds	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>S. epidermidis</i>	<i>M. luteus</i>	<i>B. cereus</i>
<b>3a</b>	20	14	12	20	18
<b>3b</b>	17	15	15	12	20
<b>3c</b>	13	15	16	17	18
<b>3d</b>	-	12	18	12	15
<b>3e</b>	-	13	19	15	15
<b>3f</b>	-	12	13	-	12
<b>3g</b>	-	-	19	12	15
<b>3h</b>	-	12	12	15	15
<b>3i</b>	20	-	12	18	11
<b>4a</b>	16	12	11	15	-
<b>4b</b>	22	19	16	20	14
<b>4c</b>	15	12	14	13	16
<b>4e</b>	-	11	20	18	12
<b>4f</b>	-	13	18	13	14
<b>4h</b>	-	15	14	13	13
*K	25	15	-	-	-
*Amp	30	16	-	-	-
*C	25	18	-	-	-
*Amc	30	22	-	-	-
*Ofx	-	-	-	-	30
*Sbc	-	-	-	-	-
Control (DMSO)	-	-	-	-	-

\*: K: Kanamycin, Amp: Ampicillin, C: Chloramphenicol, Amc: Amoxicillin, Ofx: Ofloxacin and Sbc: Sulbactam

**Table 2.** Antimicrobial activities of compounds *cis*-isomers (**3a-3i**) and *trans*-isomers (**4a-4c**, **4e-4f**, **4h**) against Gram-negative bacteria and a yeast strain

Inhibition zone diameter (mm)						
Compounds	<i>S. dysenteriae</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>B. abortus</i>	<i>P. putida</i>	<i>C. albicans</i>
<b>3a</b>	20	19	20	15	22	20
<b>3b</b>	19	21	13	22	21	21
<b>3c</b>	20	16	13	18	23	18
<b>3d</b>	16	-	-	20	15	17
<b>3e</b>	16	16	13	21	17	14
<b>3f</b>	24	20	12	21	15	21
<b>3g</b>	13	15	16	12	-	24
<b>3h</b>	23	-	15	21	16	22
<b>3i</b>	22	17	15	21	22	20
<b>4a</b>	16	16	12	20	22	15
<b>4b</b>	20	16	18	22	23	20
<b>4c</b>	19	16	-	14	16	20
<b>4e</b>	17	22	12	18	-	24
<b>4f</b>	17	22	13	17	15	22
<b>4h</b>	19	15	15	21	15	23
*K	16	25	20	-	14	-
*Amp	11	10	11	-	8	-
*C	20	30	19	-	12	-
*Amc	17	14	19	-	15	-
*Ofx	-	-	-	-	-	-
*Sbc	-	-	-	12	-	-
*Nys	*NS	*NS	*NS	*NS	*NS	20
Control (DMSO)	-	-	-	-	-	-

\*: K: Kanamycin, Amp: Ampicillin, C: Chloramphenicol, Amc: Amoxicillin, Ofx: Ofloxacin and Sbc: Sulbactam, Antifungal; Nys: Nystatin, NS: Not Studied

In a remarkable study, enhanced antifungal activity was observed in *E*-stereoisomer of isoxazolidine with >96% ee when compared to isoxazolidine with >69% ee [38]. In this study, although all the synthesized isoxazolidine compounds exhibited antibacterial activities, their effectiveness varied considerably. According to the Kruskal Wallis test result, there is a statistically significant difference among the standart drugs, *cis*-isomers and *trans*-isomers groups for antibacterial activity against *S. aureus* ve *L. monocytogenes* (p values: 0.029, 0.046 in Table 3). However, no statistical differences were found for antibacterial activity of *cis*-isomers and *trans*-isomers groups against *S. epidermidis*, *M. luteus* and *B. cereus* (p values: 0.864, 0.755 and 0.240 in Table 3). In the post-hoc test results against *S. aureus* and *L. monocytogenes* bacteria, statistically significant difference was found only between the standart drugs and *cis*-isomers groups by Bonferroni corrected Mann Whitney U test. For these bacteria, antibacterial activity of the standart drugs is higher than that of *cis*-isomers.

When the standart drugs, *cis*-isomers and *trans*-isomers were compared by Kruskal-Wallis test in terms of antibacterial activity against *S. dysenteriae*, *E. coli*, *S. typhi* and *P. putida*, against only *P. Putida*, statistically significant differences were found between three groups (p values:0.364, 0.998, 0.399 and 0.021 in Table 4). However, no statistical differences were found for antibacterial activity of *cis*-isomers and *trans*-isomers groups against *B. abortus* and *C. albicans* (p values: 0.689 and 0.529 in Table 4). Within the post-hoc test results against *P. putida*, a statistically significant difference was found only between standart drug and *cis*-isomers groups by Bonferroni corrected Mann Whitney U test results. For *P. putida*, antibacterial activity of *cis*-isomers was higher than that of standart drugs.



On the basis of above statements, it can not be deduced that the difference in antibacterial activity may be due to the stereochemical structural difference between two diastereomers which might play a role in exhibiting biological activity.

**Table 3.** Comparison of antimicrobial activity between *cis*-isomers (3a-3i), *trans*-isomers (4a-4c, 4e-4f, 4h) and standard drugs against Gram-positive bacteria

Gram-positive bacteria	Standard drugs	<i>cis</i> -isomers	<i>trans</i> -isomers	p value
<i>S. aureus</i>	27.5 (25-30)	18.5 (13-20)	16.0 (15-22)	<b>0.029*</b>
<i>L. monocytogenes</i>	17.0 (15-22)	13.0 (12-15)	12.5 (11-19)	<b>0.046*</b>
<i>S. epidermidis</i>	-	15.0 (12-19)	15.0 (11-20)	0.864 <sup>#</sup>
<i>M. luteus</i>	-	15.0 (12-20)	14.0 (13-20)	0.755 <sup>#</sup>
<i>B. cereus</i>	-	15.0 (11-20)	14.0 (12-16)	0.240 <sup>#</sup>

\*: Kruskal Wallis test; #: Mann Whitney U test

**Table 4.** Comparison of antimicrobial activity between *cis*-isomers (3a-3i), *trans*-isomers (4a-4c, 4e-4f, 4h) and standard drugs against Gram-negative bacteria and *C. albicans*

Gram-negative bacteria and <i>C. albicans</i>	Standard drugs	<i>cis</i> -isomers	<i>trans</i> -isomers	p value
<i>S. dysenteriae</i>	16.5 (11-20)	20.0 (13-24)	18.0 (16-20)	0.364*
<i>E. coli</i>	19.5 (10-30)	17.0 (15-21)	16.0 (15-22)	0.998*
<i>S. typhi</i>	19.0 (11-20)	14.0 (12-20)	13.0 (12-18)	0.399*
<i>B. abortus</i>	-	21.0 (12-22)	19.0 (14-22)	0.689 <sup>#</sup>
<i>P. putida</i>	13.0 (8-15)	19.0 (15-23)	16.0 (15-23)	<b>0.021*</b>
<i>C. albicans</i>	-	20.0 (14-24)	21.0 (15-24)	0.529 <sup>#</sup>

\*: Kruskal Wallis test; #: Mann Whitney U test

#### 4. CONCLUSION

Several isoxazolidine derivatives were synthesized in moderate-to-low yields by a two-step pathway and then, antibacterial and antifungal activities of the synthesized compounds were investigated. All the synthesized compounds were mostly effective against bacteria and a yeast strain, *C. albicans*. They were generally effective against *S. epidermidis*, *M. luteus*, *B. cereus*, *B. abortus* and *C. albicans*, while standard antibiotics kanamycin, ampicillin and chloramphenicol did not displayed any activity against these bacterial strains. The antibacterial activity differed significantly on *S. aureus*, *L. monocytogenes* and *P. putida*.

#### ACKNOWLEDGEMENT

The authors thank to Assist. Prof. Çağatay Büyükuysal for invaluable contributions in statistical analyses.

#### CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

#### REFERENCES

- [1] Zhu, J., Lines, B.M., Ganton, M.D., Kerr, M.A., Workentin, M.S., "Efficient synthesis of isoxazolidine tethered monolayer-protected gold nanoparticles (MPGNs) via 1,3-dipolar cycloadditions under high-pressure conditions", *Journal of Organic Chemistry*, 73: 1099-1105, (2008).
- [2] Karthikeyan, K., Perumal, P.T., Etti, S., Shanmugam, G., "Diastereoselective syntheses of pyrazolyl via 1,3-dipolar cycloaddition", *Tetrahedron*, 63: 10581-10586, (2007).

- [3] Dondas, H.A., Cummins, J.E., Grigg, R., Thornton, P.M., "X=Y-ZH Systems as potential 1,3-dipoles. Part 53: Sequential nucleophilic ring opening-1,3-dipolar cycloaddition reactions of Z-oxime anions with aziridines and dipolarophiles", *Tetrahedron*, 57: 7951-7964, (2001).
- [4] Casuscelli, F., Chiaccho, U., Rescifina, A., Romeo, R., Romeo, G., Tommasini, S., Uccella, N., "Ring-opening of isoxazolidine nucleus: competitive formation of  $\alpha,\beta$ -enones and tetrahydro-1,3-oxazines", *Tetrahedron*, 51: 2979-2990, (1995).
- [5] Damodiran, M., Sivakumar, P.M., Senthil Kumar, R., Muralidharan, D., Kumar, B.V.N.P., Doble, M., Perumal, P.T., "Antibacterial activity, quantitative structure-activity relationship and diastereoselective synthesis of isoxazolidine derivatives via 1,3-dipolar cycloaddition of D-glucose derived nitrene with olefin", *Chemical Biology and Drug Design*, 74: 494-506, (2009).
- [6] Yin, Z., Zhang, J., Wu, J., Liu, C., Sioson, K., Devany, M., Hu, C., Zheng, S., "Double hetero-Michael addition of N-substituted hydroxylamines to quinone monoketals: synthesis of bridged isoxazolidines", *Organic Letters*, 15: 3534-3537, (2013).
- [7] Ali, A.S., Khan, J.H., Wazeer, M.I.M., "The regiochemistry and stereochemistry of 1,3-dipolar cycloaddition of cyclic nitrenes", *Tetrahedron*, 44: 5911-5920, (1998).
- [8] Jäger, V., Müller, I., "Synthesis of amino sugars via isoxazolines: nitrile oxide-furan adducts as key intermediates in the isoxazoline route towards novel amino sugar derivatives", *Tetrahedron*, 41: 3519-3528, (1985).
- [9] Merino, P., Franco, S., Merchan, F.L., Tejero, T., "Asymmetric synthesis of an isoxazolidine nucleoside analog of thymine polyoxin C", *Tetrahedron Letters*, 39:6411-6414, (1988).
- [10] Nora, G.P., Miller, M.J., Möllmann, U., "The synthesis and in vitro testing of structurally novel antibiotics derived from acylnitroso Diels-Alder adducts", *Bioorganic and Medicinal Chemistry Letters*, 16: 3966-3970, (2006).
- [11] Litvinovskaya, R.P., Koval, N.V., Khripach, V.A., "Synthesis of modified vitamin D precursors", *Chemistry of Heterocyclic Compounds*, 36: 190-194, (2000).
- [12] Hanselmann, R., Zhou, J., Ma, P., Confalone, P.N., "Synthesis of cyclic and acyclic  $\beta$ -amino acids via chelation-controlled 1,3-dipolar cycloaddition", *Journal of Organic Chemistry*, 68: 8739-8741, (2003).
- [13] Chiacchio, U., Balestrieri, E., Macchi, B., Iannazzo, D., Piperino, A., Rescifina, A., Romeo, R., Saglimbeni, M., Sciortino, M.T., Valveri, V., Mastino, A., Romeo, G., "Synthesis of phosphonated carbocyclic 2'-oxa-3'-aza-nucleosides: novel inhibitors of reverse transcriptase", *Journal of Medicinal Chemistry*, 48: 1389-1394, (2005).
- [14] Palmer, G.C., Ordy, M.J., Simmons, R.D., Strand, J.C., Radov, L.A., Mullen, G.B., Kinsolving, C.R., St Giorgiev, V., Mitchell, J.T., Allen, S.D., "Selection of orally active antifungal agents from 3,5-substituted isoxazolidine derivatives based on acute efficacy-safety profiles", *Antimicrobial Agents and Chemotherapy*, 33:895-905, (1989).
- [15] Kumar, K.R.R., Mallesha, H., Basappa Rangappa, K.S., "Synthesis of novel isoxazolidine derivatives and studies for their antifungal properties", *European Journal of Medicinal Chemistry*, 38: 613-619, (2003).
- [16] Zhao, C., Casida, J.E., "Insect  $\gamma$ -aminobutyric acid receptors and isoxazoline insecticides: toxicological profiles relative to the binding sites of [ $^3$ H]fluralaner, [ $^3$ H]-4'-ethynyl-4-n-

- propylbicycloorthobenzoate, and [<sup>3</sup>H]ivermectin”, *Journal of Agricultural and Food Chemistry*, 62: 1019-1024, (2014).
- [17] Rakesh, D.S., Lee, R.B., Tangallapally, R.P., Lee, R.E., “Synthesis, optimization and structure-activity relationships of 3,5-disubstituted isoxazolines as new anti-tuberculosis agents”, *European Journal of Medicinal Chemistry*, 44: 460-472, (2009).
- [18] Habeeb, A.G., Rao, P.P.N., Knaus, E.E., “Design and synthesis of 4,5-diphenyl-4-isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and antiinflammatory activity”, *Journal of Medicinal Chemistry*, 44: 2921-2927, (2001).
- [19] Mondal, P., Jana, S., Balaji, A., Ramakrishna, R., Kanthal, L.K., “Synthesis of some new isoxazoline derivatives of chalconised indoline 2-one as a potential analgesic, antibacterial and anthelmintic agents”, *Journal of Young Pharmacists*, 4: 38-41, (2012).
- [20] Pinto, D.J.P., Smallheer, J.M., Cheney, D.L., Knabb, R.M., Wexler, R.R., “Factor Xa inhibitors: Next-generation antithrombotic agents”, *Journal of Medicinal Chemistry*, 53: 6243-6274, (2010).
- [21] Giofrè, S.V., Romeo, R., Carnovale, C., Mancuso, R., Cirimi, S., Navarra, M., Garozzo, A., Chiacchio, M.A., “Synthesis and biological properties of 5-(1*h*-1,2,3-triazol-4-yl) isoxazolidines: a new class of C-nucleosides”, *Molecules*, 20: 5260-5275, (2015).
- [22] Büyükgüngör, O., Yavuz, S., Odabaşoğlu, M., Özkan, H., Pamir, Ö., Yıldırım, Y., “Dimethyl trans-3-(4-bromophenyl)-2-methylisoxazolidine-4,5-dicarboxylate”, *Acta Crystallographica*, E65: o2207, (2009).
- [23] Andrade, M., Barros, M.T., Pinto, R.C., “Exploiting microwave-assisted neat procedures: synthesis of N-aryl and N-alkylnitrones and their cycloaddition en route for isoxazolidines”, *Tetrahedron*, 64: 10521-10530, (2008).
- [24] Colacino, E., Nun, P., Colacino, F.M., Martinez, J., Lamaty, F., “Solvent-free synthesis of nitrones in a ball-mill”, *Tetrahedron*, 64: 5569-5576, (2008).
- [25] Wagner, G., Garland, T., “Synthesis of 5-trichloromethyl- $\Delta^4$ -1,2,4-oxadiazolines and their rearrangement into formamidine derivatives”, *Tetrahedron Letters*, 49: 3596-3599, (2008).
- [26] Yijima, C., Tsujimoto, T., Suda, K., Yamauchi, M., “<sup>13</sup>C and <sup>1</sup>H NMR of  $\alpha$ -arylnitrones. Substituent effects on the  $\alpha$ -position of  $\alpha$ -(*p*-substituted phenyl)nitrones”, *Bulletin of the Chemical Society of Japan*, 59: 2165-2170, (1986).
- [27] Odabaşoğlu, M., Özkan, H., Yıldırım, Y., Büyükgüngör, O., “Dimethyl cis-2-methyl-3-*p*-tolylisoxazolidine-4,5-dicarboxylate”, *Acta Crystallographica*, E65: o806-o807, (2009).
- [28] Wikler, M.A., Cockerill, F.R., Craig, W.A., Dudley, M.N., Eliopoulos, G.M., Hecht, D.W., Hindler, J.F., Ferraro, M.J., Swenson, J.M., Low, D.E., Sheehan, D.J., Tenover, F.C., Turnidge, J.D., Weinstein, M.P., Zimmer, B.L., “Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard”, *Clinical and Laboratory Standards Institute*, 26: M7-A7, (2006).
- [29] Wikler, M.A., Cockerill, F.R., Craig, W.A., Dudley, M.N., Eliopoulos, G.M., Hecht, D.W., Hindler, J.F., Ferraro, M.J., Swenson, J.M., Low, D.E., Sheehan, D.J., Tenover, F.C., Turnidge, J.D., Weinstein, M.P., Zimmer, B.L., “Reference method for broth dilution antifungal susceptibility testing of yeasts approved standard”, *Clinical and Laboratory Standards Institute*, 0(0): M27-A3, Replaces, 22(15): M27-A2, (2007).

- [30] Iannazzo, D., Brunaccini, E., Giofre, S., Piperno, A., Romeo, G., Ronsisvalle, S., Chiacchio, M.A., Lanza, G., Chiacchio, U., "Competitive formation of  $\beta$ -enaminones and 3-amino-2(5*H*)-furanones from the isoxazolidine system: a combined synthetic and quantum chemical study", *European Journal of Organic Chemistry*, 2010:5897-5905, (2010).
- [31] Alavi Nikje, M.M., Bigdeli, M.A., Imanieh, H. A., "Facile and new method for the synthesis of  $\alpha$ -aryl-*N*-methylnitrones in solvent-free media using silica gel-NaOH", *Phosphorus, Sulfur, and Silicon and the Related Elements*, 179:1465-1468, (2004).
- [32] Yavuz, S., Ozkan, H., Colak, N., Yildirim, Y., "Fast method for synthesis of alkyl and aryl-*N*-methylnitrones", *Molecules*, 16:6677-83, (2011).
- [33] Marvaniya, H.M., Modi, K.N., Sen, D.J., "Greener reactions under solvent free conditions", *International Journal of Drug Development and Research*, 3:34-43, (2011).
- [34] Zangade, S.B., Mokle, S.S., Shinde, A.T., Vibhute, Y.B., "An atom efficient, green synthesis of 2-pyrazoline derivatives under solvent-free conditions using grinding technique", *Green Chemistry Letters and Reviews*, 6: 123-127, (2013).
- [35] Singh, G., Sharma, A., Kaur, H., Ishar, M.P.S., "Chromanyl-isoxazolidines as antibacterial agents: synthesis, biological evaluation, quantitative structure activity relationship, and molecular docking studies", *Chemical Biology and Drug Design*, 87: 213-223, (2016).
- [36] Chacrabarty, B., Samanta, A., Sharma, P.K., Singh, C., Kafley, S., Banerjee, A., Sinha, C., "One pot high yield synthesis of some novel isoxazolidine derivatives using *N*-methyl- $\alpha$ -chloro nitron in water and their antibacterial activity", *Journal of Chemical and Pharmaceutical Research*, 2: 727-739, (2010).
- [37] Chacrabarty, B., Chhetri, M.S., Kafley, S., Samanta, A., "Synthesis and antibacterial activities of some novel isoxazolidine derivatives derived from *N*-phenyl- $\alpha$ -chloro nitron in water", *Indian Journal of Chemistry*, 49B: 209-215, (2010).
- [38] Sadashiva, M.P., Mallesha, H., Karunakara Murthy, K., Rangappa, K.S., "Enhancement in antimicrobial activity of 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine", *Bioorganic and Medicinal Chemistry Letters*, 15: 1811-1814, (2005).
- [39] Kumar, K.R.R., Mallesha, H., Rangappa, K.S., "The synthesis of novel isoxazolidine derivatives and their antifungal and antibacterial properties", *Archiv der Pharmazie-Pharmaceutical and Medicinal Chemistry*, 336: 159-164, (2003).
- [40] Kumar, K.R.R., Mallesha, H., Rangappa, K.S., "Synthesis and characterization of 5-substituted novel isoxazolidines derived from 1,3-dipolar cycloaddition of nitrones with olefins: studies of antibacterial and antifungal activities", *Synthetic Communications*, 33: 1545-1555, (2003).
- [41] Berthet, M., Cheviet, T., Dujardin, G., Parrot, I., Martinez, J., "Isoxazolidine: a privileged scaffold for organic and medicinal chemistry", *Chemical Reviews*, 116: 15235-15283, (2016).
- [42] Źelechowski, K., Gołębiewski, W.M., "Synthesis and fungicidal activity of 2-diphenylmethyl 3-arylisoxazolidine-5-carboxamides", *Monatshefte für Chemie*, 146: 1895-1905, (2015).