Potassium carbonate mediated one-pot synthesis and antimicrobial activities of 2-alkoxy-4-(aryl)-5H-indeno[1,2-b]pyridine-3-carbonitriles

Şahin Öztürk1, Meliha Burcu Gürdere1, Hayreddin Gezegen2, Mustafa Ceylan1 and Yakup Budak*1

1Department of Chemistry, Faculty of Arts and Sciences, Gaziosmanpasa University, 60250, Tokat, Türkiye
2Department of Nutrition and Dietetics, Faculty of Health Science, Cumhuriyet University, Sivas, Türkiye

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Abstract. 2-Alkoxyl-4-(aryl)-5H-indeno[1,2-b]pyridine-3-carbonitriles (3a-e and 4a-e) were synthesized via multicomponent reaction from 2-aryl-methylidinedindan-1-ones (1a-e), malononitrile and K2CO3 in ethanol and/or methanol. The structures of obtained compounds (3a-e and 4a-e) were characterized using the spectroscopic methods (NMR, IR) and elemental analysis. Addition, the in vitro antimicrobial activities of compounds (3a-e) were tested against the five human pathogenic bacteria. Penicillin G and Ceftriaxone antibiotics were used as positive control. The results were given as MIC values (minimum inhibition concentration), and compounds 3b-d showed very high activity against Escherichia coli 111.

Keywords: 1-Indanone; malononitrile; pyridine-3-carbonitrile; antimicrobial activity. © 2016 ACG Publications. All rights reserved.

1. Introduction

Carbocyclic compounds containing an aromatic moiety, such as indanones, are an important component of compounds exhibiting pharmacological properties. Indanone derivatives are used as drugs in the treatment of diseases such as cancer and Alzheimer's disorders.1 The indanones are also used as drug intermediates, as ligands of olefinic polymerization catalysts and as discotic liquid crystals.1,2 On the other hand, pyridine and its derivatives are the most popular N-heteroaromatics used in the chemical industry for the production of stain, pesticide and pharmaceutical products,3 and many different derivatives are known to exhibit biological activity.4 Cyanopyridines containing different aryl and alkyl groups have also been found to possess properties such as antimicrobial,5 antihypertensive,6 cardiovascular,7 antipyretic,8 analgesic,9 antitumor10 and IKK-β inhibitors.11 For the synthesis of these type compounds, several methods have been developed.11-14 One of these methods is multicomponent reactions which are very usefulness for the synthesis of heterocyclic compounds.15-18 Tyndall et al.19

*Corresponding author: E-Mail: yakup.budak@gop.edu.tr, Tel: +90 3562521616; fax: +90 3562521585.
have applied this method to 1,3-diphenylpropane for the synthesis of 1,4-diphenyl-2-methoxypyridine-3-carbonitriles. Furthermore, they have synthesized the 2-Methoxy-4-phenyl-5H-indeno[1,2-b]pyridine-3-carbonitrile (4e) from the one-pot reaction of 2-benzylidene-2,3-dihydro-1H-inden-1-one, malononitrile and NaOH in CH$_2$OH in yield of 50%. Then, Mishriky et al. have synthesized some 5H-indeno[1,2-b]pyridine derivatives (3a, c, e and 4a, c, e) with the same method using the KOH instead of NaOH.

Our previous work, the 2-(1,3-dihetaryl)-3-oxopropyl)malononitrile derivatives were submitted to K$_2$CO$_3$ prompted cyclization reaction for the synthesis of 4,6-dihetaryl-2-alkoxy(pyridine-3-carbonitriles. The reactions were performed in methanol and/or ethanol at reflux temperature and desired compounds were obtained in high yields. Encouraged by the above-mentioned findings and in the continuation of our ongoing research in the field of synthesis and biological screening of nitrogen containing heterocycles we mentioned findings and in the continuation of our ongoing research in the field of synthesis and biological screening of nitrogen containing heterocycles we

2. Experimental

IR spectra (KCl disc) were recorded on a Jasco FT/IR-430 spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance DPX-400 instrument. As internal standards served TMS ($\delta$ 0.00) for $^1$H NMR and CDCl$_3$ ($\delta$ 77.0) for $^{13}$C NMR spectroscopy J values are given in Hz. Melting points were measured on Electrothermal 9100 apparatus. Elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer. The major chemicals were purchased from Sigma-Aldrich and Fluka.

2.1. Chemistry

General Procedure for the Synthesis of 2-Alkoxypyridine derivatives (3a-e and 4a-e): 2-Aryl-methylidineindan-1-ones (1a-e) (0.80 mmol) were dissolved in ethanol and/or methanol. Malononitrile (2) (0.95 mmol) and K$_2$CO$_3$ (3.2 mmol) was added to the mixture and stirred at reflux temperature for 16 hours followed by acidification with 5% HCl. The reaction mixture was extracted with CHCl$_3$ and/or CH$_2$Cl$_2$ (20 mL), dried over Na$_2$SO$_4$, the solvent removed under reduced pressure. The solid crude product was purified on a short silica gel column eluting with chloroform-hexane (3:1), and the obtained solid was recrystallized from chloroform-hexane (2:1) and/or ethanol-diethyl ether (2:1).

2-Ethoxy-4-(4-methoxphenyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (3a): Yellowish crystals, Yield, 82%, M.P. 170-173 C (lit.$^{20}$ 170-172 C). IR (KCl, cm$^{-1}$): 2979, 2937, 2904, 2834, 2215, 1698, 1608, 1583, 1558, 1517, 1484, 1438, 1375, 1336, 1294, 1253, 1186, 1155, 1033, 929, 838, 767, 730, 649, 590, 559, 518, 420. $^1$H-NMR (400 MHz, CDCl$_3$, ppm): $\delta$ 8.08-8.06 (m, 1H), 7.57-7.52 (m, 3H), 7.51-7.46 (m, 2H), 7.09 (d, $J = 8.4$ Hz, 2H), 4.70 (q, $J = 7.2$ Hz, 2H), 3.91 (s, 3H), 3.78 (s, 2H), 1.55 (t, $J = 7.0$ Hz, 3H). $^1$H-NMR data is agreement with data given in the literature.$^{20}$

$^{13}$C-NMR (100 MHz, CDCl$_3$, ppm): $\delta$ 166.0, 161.5, 160.0, 151.7, 145.5, 140.0, 130.1 (2C), 129.8, 127.6, 127.4, 127.3, 125.1, 121.9, 116.5, 114.3 (2C), 92.5, 63.3, 55.5, 34.0, 14.6. Anal. calc. for C$_{29}$H$_{18}$N$_2$O$_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.08; H, 5.12; N, 8.11.

2-Ethoxy-4-(p-tolyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (3b): Yellowish crystals, Yield, 73%, M.P. 160-163 C. IR (KCl, cm$^{-1}$): 2983, 2906, 2360, 2217, 1716, 1654, 1616, 1556, 1515, 1376, 1340, 1193, 1149, 1031, 923, 825, 763, 651, 503, 418. $^1$H-NMR (400 MHz, CDCl$_3$, ppm): $\delta$ 8.09-8.07 (m, 1H), 7.56-7.54 (m, 1H), 7.51-7.46 (m, 4H), 7.37 (d, $J = 8.0$ Hz, 2H), 4.70 (q, $J = 7.2$ Hz, 2H), 3.78 (s, 3H), 2.49 (s, 3H), 1.55 (t, $J = 7.0$ Hz, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$, ppm): $\delta$ 165.9, 161.5, 152.0, 145.5, 139.9, 139.6, 132.2, 129.8, 129.5 (2C), 128.4 (2C), 128.3, 127.6, 127.4, 125.1, 121.9, 92.6,
63.3, 33.9, 21.5, 14.6. Anal. calc. for C_{22}H_{16}N_{2}O: C, 80.96; H, 5.56; N, 8.58. Found: C, 80.88; H, 5.48; N, 8.49.

2-Ethoxy-4-(4-chlorophenyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (3c): Colorless crystals, Yield, 78%, M.P. 213-216°C, (lit.20 213-214°C). IR (KCl, cm⁻¹): 2983, 2904, 2358, 2217, 1556, 1484, 1440, 1334, 1197, 1153, 1091, 1033, 927, 831, 765, 728, 644, 499, 420.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.11-8.08 (m, 1H), 7.59-7.53 (m, 3H), 7.52-7.48 (m, 4H), 4.71 (q, J = 7.2 Hz, 2H), 3.76 (s, 2H), 1.55 (t, J = 7.0 Hz, 3H).¹3C-NMR data is agreement with data given in the literature.²⁰

3d: Yellowish crystals, Yield, 76%, M.P. 188-191°C. IR (KCl, cm⁻¹): 2987, 2215, 1698, 1558, 1475, 1419, 1373, 1332, 1274, 1236, 1155, 1033, 892, 763, 732, 620, 543, 491, 420.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.11-8.08 (m, 1H), 7.23-7.15 (m, 2H), 7.58-7.56 (m, 1H), 7.52-7.48 (m, 3H), 7.47 (br d, J = 7.6 Hz, 1H), 4.72 (q, J = 7.0 Hz 2H), 3.76 (s, 2H), 1.55 (t, J = 7.0 Hz, 3H).¹³C-NMR (100 MHz, CDCl₃, ppm): δ 165.9, 161.8, 150.0, 145.5, 139.7, 137.1 132.6, 131.3, 130.5 (2C), 127.5, 127.2 (2C), 125.2, 122.8, 122.0, 115.7, 92.4, 63.5, 33.6, 14.6. Anal. calc. for C_{23}H_{15}ClN_{2}O: C, 64.46; H, 3.86; N, 7.16. Found: C, 64.37; H, 3.75; N, 7.09.

2-Ethoxy-4-(3-bromophenyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (3d): Yellowish crystals, Yield, 81%, M.P. 186-189°C, (lit.20 187-189°C). IR (KCl, cm⁻¹): 3056, 2983, 2362, 2219, 1868, 1558, 1506, 1436, 1375, 1334, 1240, 1155, 1024, 763, 721, 698, 620, 482, 420.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.11-8.06 (m, 1H), 7.57-7.54 (m, 6H), 7.51-7.47 (m, 2H), 4.72 (q, J = 7.0 Hz, 2H), 3.78 (s, 2H), 1.56 (t, J = 7.0 Hz, 3H).¹³C-NMR data is agreement with data given in the literature.²⁰

2-Ethoxy-4-(phenyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (3e): Yellowish crystals, Yield, 81%, M.P. 186-189°C, (lit.20 187-189°C). IR (KCl, cm⁻¹): 3056, 2983, 2362, 2219, 1868, 1558, 1506, 1436, 1375, 1334, 1240, 1155, 1024, 763, 721, 698, 620, 482, 420.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.11-8.06 (m, 1H), 7.57-7.54 (m, 6H), 7.51-7.47 (m, 2H), 4.72 (q, J = 7.0 Hz, 2H), 3.78 (s, 2H), 1.56 (t, J = 7.0 Hz, 3H).¹³C-NMR data is agreement with data given in the literature.²⁰

2-Methoxy-4-(4-methoxyphenyl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (4a)²⁰: Yellowish crystals, Yield, 80%, M.P. 181-184°C, (lit.20 195-197°C). IR (KCl, cm⁻¹): 3052, 2994, 2950, 2221, 1924, 1606, 1552, 1482, 1455, 1355, 1290, 1241, 1186, 1025, 952, 842, 763, 728, 646, 590, 516, 489, 422.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.10 (dd, J = 6.2, 2.2 Hz, 1H), 7.57-7.52 (m, 3H), 7.50-7.47 (m, 2H), 7.08 (d, J = 8.8 Hz, 2H), 4.23 (s, 3H), 3.91 (s 3H), 3.80 (s, 2H).¹³C-NMR data is agreement with data given in the literature.²⁰

2-Methoxy-4-(p-tolyloxy)-5H-indeno[1,2-b]pyridine-3-carbonitrile (4b): Yellowish crystals, Yield, 70%, M.P. 171-187°C, IR (KCl, cm⁻¹): 2954, 2356, 2219, 1691, 1625, 1558, 1484, 1361, 1272, 1207, 1149, 1010, 956, 823, 767, 730, 646, 520, 484, 418.¹H-NMR (400 MHz, CDCl₃, ppm): δ 8.10 (br d, J = 7.4 Hz, 1H), 7.55 (br d, J = 7.2 Hz, 1H), 7.51-7.43 (m, 4H), 3.37 (d, J = 8.0 Hz, 2H), 4.24 (s, 3H), 3.79 (s, 2H), 2.48 (s, 3H).¹³C-NMR (100 MHz, CDCl₃, ppm): δ 166.2, 161.5, 152.0, 145.6, 139.9, 139.7, 132.1, 130.8, 129.8, 129.5, 128.4, 127.9, 127.4, 125.1, 122.0, 92.5, 54.6, 33.9, 21.5. Anal. calc. for C_{21}H_{16}N_{2}O: C, 80.75; H, 5.16; N, 8.97. Found: C, 80.68; H, 5.09; N, 8.87.

4-(4-Chlorophenyl)-2-methoxy-5H-indeno[1,2-b]pyridine-3-carbonitrile (4c)²⁰: Yellowish crystals, Yield, 81%, M.P. 236-239°C, (lit.20 237-239°C). IR (KCl, cm⁻¹): 2983, 2946, 2360, 2225, 1868, 1579,
Antimicrobial activities of 2-alkoxy-4-(aryl)-5H-indeno[1,2-b]pyridine-3-carbonitriles

1556, 1484, 1457, 1361, 1280, 1149, 1091, 1008, 954, 831, 761, 725, 725, 638, 638, 482, 420. \(^1\)H-NMR (400 MHz, CDCl\(_3\), ppm): \(\delta 8.12-8.07\) (m, 1H), 7.58-7.53 (m, 3H), 7.52-7.47 (m, 4H), 4.23 (s, 3H), 3.74 (s, 2H). \(^1\)H-NMR data is agreement with data given in the literature.\(^{20}\)

\(^{13}\)C-NMR (100 MHz, CDCl\(_3\), ppm): \(\delta 166.2, 161.9, 150.6, 145.5, 139.7, 135.9, 133.4, 130.2, 129.9\) (2C), 129.3 (2C), 127.7, 127.6, 125.2, 122.1, 115.9, 92.3, 54.7, 33.7. Anal. calc. for \(\text{C}_{20}\text{H}_{13}\text{ClN}_{2}O\): C, 72.18; H, 3.94; N, 8.42. Found: C, 72.14; H, 3.86; N, 8.34.

4-(3-Bromophenyl)-2-methoxy-5H-indeno[1,2-b]pyridine-3-carbonitrile (4d): Yellowish crystals, Yield, 84\%, M.P. 183-185°C. IR (KCl, cm\(^{-1}\)): 3058, 2952, 2360, 2223, 1637, 1556, 1475, 1405, 1359, 1276, 1240, 1211, 1153, 1074, 869, 798, 765, 619, 485, 420. \(^1\)H-NMR (400 MHz, CDCl\(_3\), ppm): \(\delta 8.13-8.09\) (m, 1H), 7.71-7.66 (m, 2H), 7.58-7.56 (m, 1H), 7.52-7.48 (m, 3H), 7.45 (t, \(J = 8.0\) Hz, 1H), 4.25 (s, 3H), 3.77 (s, 2H). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\), ppm): \(\delta 166.2, 161.9, 150.1, 145.5, 139.7, 137.0, 132.6, 131.2, 130.5, 130.2, 127.7, 127.6, 127.2, 125.2, 122.9, 122.1, 115.7, 92.4, 54.7, 33.7. Anal. calc. for \(\text{C}_{20}\text{H}_{13}\text{BrN}_{2}O\): C, 63.68; H, 3.47; N, 7.43. Found: C, 63.54; H, 3.43; N, 7.39.

2-Methoxy-4-phenyl-5H-indeno[1,2-b]pyridine-3-carbonitrile (4e)\(^{19,20}\): Yellowish crystals, Yield, 74\%, M.P. 172-175°C, (lit.\(^{20}\) 143-144°C). IR (KCl, cm\(^{-1}\)): 3058, 2948, 2223, 1965, 1635, 1558, 1484, 1359, 1278, 1211, 1174, 1020, 860, 765, 727, 696, 663, 617, 522, 470, 420. \(^1\)H-NMR (400 MHz, CDCl\(_3\), ppm): \(\delta 8.11-8.09\) (m, 1H), 7.61-7.53 (m, 6H), 7.52-7.44 (m, 2H), 4.24 (s, 3H), 3.77 (s, 2H). \(^1\)H-NMR data is agreement with data given in the literature.\(^{20}\)

\(^{13}\)C-NMR (100 MHz, CDCl\(_3\), ppm): \(\delta 166.2, 161.5, 152.0, 145.5, 139.8, 135.0, 130.0, 129.6, 128.9\) (2C), 128.5 (2C), 127.9, 127.5, 125.1, 122.1, 116.1, 92.4, 55.4, 34.3. Anal. calc. for \(\text{C}_{20}\text{H}_{12}\text{N}_{2}O\): C, 80.52; H, 4.73; N, 9.39. Found: C, 80.46; H, 4.66; N, 9.24.

2.2 Biological Activity Assay

All newly synthesized compounds 3a-e were screened for their \textit{in vitro} antimicrobial activities by disc-diffusion method using Mueller-Hilton agar medium against \textit{Staphylococcus aureus} ATCC 29213 and \textit{β-Hemolytic Streptococcus} ATCC 2957, \textit{Escherichia coli} 111, \textit{Salmonella Enteritidis} ATCC 13076 and \textit{Klebsiella pneumoniae} ATCC 1383. The results were given as MIC values (minimum inhibition concentration) compared with positive control (Penicillin G and Ceftriaxone antibiotics).\(^{22,24}\)

3. Results and discussion

3.1 Chemistry

In this work, the synthesis of (2-alkoxy-4-aryl-5H-indeno[1,2-b] pyridine-3-carbonitrile) derivatives (3a-e and 4a-e) containing both the indane and the pyridine ring in a single structure was targeted. For this, firstly known 2-aryl-methylidineindan-1-ones (1a-e) were synthesize according to published methods.\(^{25,28}\) The multicomponent (one-pot) reaction of 2-aryl-methylidineindan-1-ones (1a-e) malononitrile and K\(_2\)CO\(_3\) in EtOH and/or MeOH at reflux temperature for 16 hours gave the (2-alkoxy-4-aryl-5H-indeno[1,2-b] pyridine-3-carbonitrile) derivatives (3a-e and 4a-e) in good yields (74\%-84\%) (Figure 1, Table 1).
Among the synthesized compounds, six compounds (3a, c, e and 4a, c, e) were previously synthesized by Mishriky et al.\textsuperscript{20} from the one-pot reaction of 2-aryl-methyldieneindan-1-ones, malononitrile and KOH in EtOH and/or MeOH at room temperature for 24 hours. They reported that the compounds (3a, c, e and 4a, c, e) were obtained in moderate yields (40\%-54\%) with the formation of 2-(2-aryl-2,3-dihydro-1H-inden-1-ylidene)malononitriles, as side products. In our present procedure, the desired compounds were obtained higher yields (74\%-84\%) without formation of by-products. The structures of all synthesized compounds were determined on the basis of spectral data (NMR and IR) and comparison with their authentic samples.\textsuperscript{19,20} The \textsuperscript{1}H-NMR data of compounds are in good agreement.
with data of reported by Mishriky et al.\textsuperscript{20} Furthermore, the most decisive signal $^1$C-MR spectra of 3a-3e and 4a-e is the presence of a signal in the region at $\delta$ 116-114 ascribable to CN. Another noteworthy feature is the appearance of a signal in the region at $\delta$ 93-92 which was assigned to C3 as evidenced by literature in similar structures.\textsuperscript{29,30} Addition, all physical and spectral data were in good agreement with proposed structures.

3.2. Antimicrobial activity

The synthesized compounds (3a-e) were tested for their antibacterial activity against five different types of human pathogenic bacterial strains. Used microorganisms were: Staphylococcus aureus ATCC 29213 and $\beta$-Hemolytic Streptococcus ATCC 2957 which are Gram-positive bacteria and Escherichia coli 111, Salmonella Enteritidis ATCC 13076 and Klebsiella pneumoniae ATCC 13883 which are Gram-negative bacteria. In these tests, P (Penicillin) and CEF (Ceftriaxone) were used as standard and DMSO was used as negative control. The results were given as MIC values (minimum inhibition concentration) compared with positive control (Penicillin G and Ceftriaxone antibiotics) (Table 2).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Microorganisms</th>
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<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus ATCC 29213</td>
</tr>
<tr>
<td>3a</td>
<td>250</td>
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<tr>
<td>3b</td>
<td>125</td>
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<td>3c</td>
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<tr>
<td>CEF</td>
<td>31.25</td>
</tr>
<tr>
<td>DMSO</td>
<td>250</td>
</tr>
</tbody>
</table>

PEN: Penicillin G; CEF: Ceftriaxone antibiotics

As seen Table 2, all compounds (3a-e) showed low activity against Staphylococcus aureus with 125-250 µg/mL MIC values compared to standards (MIC = PEN = CEF = 31.25 µg/mL). While compounds (3a-e) showed very lower activity than the standard CEF (MIC = 15.63 µg/mL) with 250 µg/mL MIC values, they exhibited higher activity than standard PEN (MIC = 500 µg/mL) against $\beta$-Hemolytic Streptococcus. Almost all compounds (3a-e) displayed very good activity against Escherichia coli 111. The MIC values of compounds (3a-e) were 125 µg/mL for 3a, 7.81 µg/mL for 3b, 3.91 µg/mL for 3c, 7.81 µg/mL for 3d and 62.25 µg/mL for 3e, whereas MIC values standard PEN and CEF were 1.95 µg/mL and 31.25 µg/mL, respectively. While compound 3a demonstrated the same activity with CEF against Salmonella Enteritidis (MIC = 62.25 µg/mL), it showed lower activity than the PEN (MIC = 0.98 µg/mL). The other compounds displayed low activity than standards. All compounds 3a-e exhibited higher activity than standards (MIC = PEN = CEF = 500 µg/mL) against Klebsiella pneumoniae with MIC values (125 µg/mL for 3a, 3b and 3e, and 250 µg/mL for 3c and 3d). According to these results, further researches can be performed for compounds 3a-d as potential antibacterial agents against Escherichia coli 111. The structure activity relationship (SAR), the most active compounds were 3c (MIC = 3.91 µg/mL) containing chlorine atom against Escherichia coli 111 and 3a (MIC = 62.25 µg/mL) containing methyl group against Salmonella Enteritidis.
4. Conclusion

The 2-Alk oxy-4-(aryl)-5H-indeno[1,2-b]pyridine-3-carbonitriles (3a-e and 4a-e) were synthesized via K$_2$CO$_3$ mediated one-pot reaction from 2-aryl-methylidineindan-1-ones (1a-e), malononitrile and K$_2$CO$_3$ in ethanol and/or methanol in high yields. Addition, the in vitro antimicrobial activities of compounds (3a-e) were tested against the five human pathogenic bacteria. Compounds 3b-d showed very high activity against *Escherichia coli* 111 with MIC 7.81 µg/mL for 3b, 3.91 µg/mL for 3c, 7.81 µg/mL for 3d and 62.25 µg/mL for 3e, compared to standards PEN and CEF (MIC = 1.95 µg/mL and 31.25 µg/mL, respectively). From these results, further researches can be performed for compounds 3a-d as potential antibacterial agents against *Escherichia coli* 111.

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