Synthesis, Characterization and \textit{in vitro} Antibacterial Activity of Novel 1,2,4-Triazine and 1,2-Diazeepine Derivatives

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Abstract: In this work we describe a simple method to synthesis novel 3-[(pyridine-2-ylamino)methyl]-1,6-dihydro-1,2,4-triazine-5(2H)-one (3) and 1-(N-pyridine-2-ylglycl)-1,2-diazeepine-3,7-dione (4). Firstly, adding ethyl chloroacetate to a solution of KOH and 2-aminopyridine and refluxed in absolute ethanol for 5 hours afforded the target ethyl-N-pyridin-2-ylglycin (1), then refluxing of compound (1) with hydrazine hydrate in absolute ethanol for 6 hours yield 2-(pyridine-2-ylamino)acetohydrazide (2). Chloroacetamid and glutaric acid were refluxed with compound (2) in absolute ethanol for 20 and 21 hours to afford the compounds 3-[(pyridine-2-ylamino)methyl]-1,6-dihydro-1,2,4-triazine-5(2H)-one (3) and 1-(N-pyridine-2-ylglycl)-1,2-diazeepine-3,7-dione (4), respectively. Structures of the synthesized compounds were supported by means of FTIR, CHN elemental analysis, and NMR ($^1$H, $^{13}$C) spectroscopic analysis. The synthesized compounds were evaluated for their \textit{in vitro} antibacterial activity against \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Staphylococcus aureus}, \textit{Bacillus cereus}, and \textit{Enterococcus faecalis}. The inhibition zones were measured, expressed in mm, and the minimum inhibitory concentration (MIC) is reported in μg/mL. The results show that compounds (3) and (4) have a significant antimicrobial activity with the highest MIC value against all tested bacteria.

Keywords: 1,2-diazeepine; 1,2,4-triazine; 2-aminopyridine; antibacterial activity.

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1. Introduction

In the past decades, the problem of multi-drug-resistant micro-organisms has reached alarming levels worldwide, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. Heterocyclic organic chemistry is one of the most important and well-studied branches of medicinal chemistry. An important feature of heterocyclic bioactive compounds is their various constituent heteroatoms, including nitrogen [1-4], sulfur [5-7], oxygen [8-10], and others [11]. These heteroatoms directly affect the reactivity of the target skeleton, activity (or toxicology) of the compounds, interactions between the target drugs and different target inhibitors, and metabolism and pharmacokinetics. Heterocyclic compounds have great applicability in the pharmaceutical industry as anticancer agents, antituberculosis agents, analgesics, hypnotics, antimalarials, antimicrobials, pesticides, and insecticides [12-15]. The range of applications of heterocyclic compounds is very wide. They are of specific importance as they are associated with a wide variety of physiological
activities. Several heterocyclic compounds that occur in nature are involved biologically, including nucleic acids (in DNA and RNA), vitamins (thiamine, B\textsubscript{1}; riboflavin, B\textsubscript{2}; nicotinamide, B\textsubscript{3}), essential amino acids (proline, histidine, and tryptophan) [16, 17] and heme (hemoglobin) [18-21].

Among heterocycles, nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development in organic synthesis [22, 23]. Nitrogen-containing heterocycles have been used as medicinal compounds for centuries and form the basis for many common drugs, such as morphine, captopril, and vincristine (cancer chemotherapy). Nitrogen-containing heterocycles occur in a diverse set of natural products, and nitrogen-containing drugs are of great importance in a wide variety of applications [24-26]. In view of these facts, we have designed the synthesis of novel heterocyclic compounds derived from 2-aminopyridine and investigation their antimicrobial activity.

2. Materials and Methods

2.1. Materials.

2-Aminopyridine, ethyl chloroacetate, abs. ethanol, hydrazine hydrate, chloroacetamide were obtained from Fluka, Aldrich, BHD, Systerm, and Merck, respectively. The other chemicals used in this work were obtained from Aldrich Chemicals Company, and they were all analytical reagents and used directly without further purification.

2.2. Synthesis of ethyl-N-pyridin-2-ylglycinate (1).

Ethyl chloroacetate (0.27 mole, 29 ml) was added dropwise to a stirred solution of 2-aminopyridine (0.27 mole, 26g), KOH (0.27 mole, 15g) in (60 ml) absolute ethanol. The reaction mixture was refluxed for 5 hours, and then it was filtered. The resulting product was dried and recrystallized from ethanol.

2.3. Synthesis of 2-(pyridin-2-ylamino)acetohydrazide (2).

A mixture of compound (1) (0.08 mole, 16g) and hydrazine hydrate (0.08 mole, 2.48 ml) was refluxed for 3 hours, ethanol (40 ml) was added, and the reaction mixture was refluxed for another 3 hours. The separated precipitate was collected and recrystallized from ethanol.

2.4. Synthesis of 3-[(pyridin-2-ylamino)methyl]-1,6-dihydro-1,2,4-triazine-5(2H)-one (3).

A mixture of compound (2) (0.012 moles, 2g) and chloroacetamide (0.012 moles, 1.12g), and (20 ml) absolute ethanol was refluxed for 20 hours. The solvent was distilled off, and the solid that separated was dried and recrystallized from ethanol.

2.5. Synthesis of 1-(N-pyridine-2-ylglycyl)-1,2-diazepine-3,7-dione (4).

Compound (2) (0.012 moles, 2g) and glutaric acid (0.012 moles, 1.58g) were heated under reflux in (20 ml) absolute ethanol for 21 hours. The excess solvent was evaporated, and the crude product was collected by filtration. The target compound was obtained through recrystallization from ethanol.
2.6. Instrumentation.

Elemental C, H, and N analyses were carried out on a Fison EA 1108 analyzer. The melting points were determined in open capillary tubes using an electrothermal 9300 digital melting point apparatus. The FTIR spectra of the synthesized compounds were recorded on Shimadzu FTIR-8300 spectrometer as KBr disc. Fourier transform Bruker spectrometer (400MHz $^1$H/ 100.61MHz $^{13}$C), relative to the internal standard tetramethylsilane (TMS), and the chemical shifts are reported in part per million (ppm) using DMSO-d$_6$ as a solvent in all experiments.

3. Results and Discussion

3.1. Synthesis of target compounds.

The compounds (3) and (4) were synthesized firstly from the reaction of starting material, 2-aminopyridine, with ethyl chloroacetate to yield ethyl-N-pyridin-2-ylglycinate (1), then compound (1) converted to 2-(pyridin-2-ylamino)acetohydrazide (2) through the reaction with hydrazine hydrate. As shown in Figure 1, reaction of compound (2) with chloroacetamide and glutaric acid afforded 3-[(pyridin-2-ylamino)methyl]-1,6-dihydro-1,2,4-triazine-5(2H)-one (3) and 1-(N-pyridine-2-ylglycyl)-1,2-diazepine-3,7-dione (4), respectively. The purity of all synthesized compounds was checked by TLC using silica gel-G as an absorbent. Their physical properties and analytical data are recorded in Table 1.

![Figure 1](https://biointerfaceresearch.com/)
Table 1. Physical properties and analytical data of the synthesized compounds.

| Comp. | Molecular formula | Molecular weight, g/mol | Color     | % Yield | M.P., °C | Found (Calcd.) % | C    | H    | N    |
|-------|------------------|-------------------------|-----------|---------|---------|-----------------|------|------|------|
| 1     | C₉H₁₂N₂O₂        | 180.09                  | White     | 97      | 242-244 | 59.99 (58.43)   | 6.71 | 15.55|      |
| 2     | C₉H₁₀N₄O        | 166.18                  | Yellow    | 85      | 220-222 | 50.59 (51.92)   | 6.07 | 33.71|      |
| 3     | C₉H₁₁N₂O        | 205.22                  | Brown     | 93      | 189-191 | 52.67 (53.39)   | 5.40 | 34.13|      |
| 4     | C₁₂H₁₄N₃O₃      | 262.26                  | Dark yellow | 89    | 200-203 | 54.96 (55.67)   | 5.83 | 21.36|      |

3.2. Fourier Transform infrared (FTIR) analysis.

Solid-state infrared spectra of the synthesized compounds are recorded in the range 4000-500 cm⁻¹. The FTIR spectrum of compound (1) showed stretching bands at 3247 cm⁻¹ and 2954 cm⁻¹ which were assigned to the (N-H) and (C-H) aliphatic stretching frequencies, respectively. Besides this, the appearances of (C=O) stretching band attributable to the ester group at 1750 cm⁻¹ is in good agreement with the structure given [27]. Besides that, the FTIR spectrum of acid hydrazide (2) showed the stretching bands at 3390-3255 cm⁻¹ which were assigned to the (-NHNH₂) group stretching frequency. The disappearance of (C=O) stretching band attributable to ester group of compound (1) at 1750 cm⁻¹ with the appearance of bands at 1645 cm⁻¹ of (Amide I) and at 1585 cm⁻¹ of (Amide II) proved the formation of compound (2) [28].

![FTIR spectrum of compound 1](image1)

![FTIR spectrum of compound 2](image2)
Figure 2. FTIR spectra of (a) ethyl-N-pyridin-2-ylglycinate (1); (b) 2-(pyridin-2-ylamino)acetohydrazide (2); (c) 3-[(pyridin-2-ylamino)methyl]-1,6-dihydro-1,2,4-triazine-5(2H)-one (3); (d) 1-(N-pyridine-2-ylglycyl)-1,2-diazepine-3,7-dione (4).

Furthermore, the FTIR spectrum of compound (3) shows bands at 3265 cm\(^{-1}\) which was assignable to (N-H) stretching vibration. The bands at 3093 cm\(^{-1}\), 2827 cm\(^{-1}\), and 1650 cm\(^{-1}\) were due to \(\nu(C-H)\) aromatic, \(\nu(C-H)\) aliphatic, and \(\nu(C=O)\) moiety of triazine ring, respectively [29]. While the FT-IR spectrum of compound (4) showed stretching bands at 3265 cm\(^{-1}\), 3064 cm\(^{-1}\), 2829 cm\(^{-1}\), and 1654 cm\(^{-1}\), assignable for (N-H), (C-H) aromatic, (C-H) aliphatic, and (C=O) groups, respectively. Figure (a), (b), (c), and (d) show the FTIR spectra of compounds (1), (2), (3), and (4), respectively.

3.3. Proton nuclear magnetic resonance (\(^1\)H NMR) analysis.

The \(^1\)H NMR spectra for the synthesized compounds (3) and (4) were recorded in d\(_6\)-DMSO using tetramethylsilane as the internal standard. \(^1\)H-NMR spectrum of 3-[(pyridine-2-ylamino) methyl]1,6-dihydro-1,2,4-triazin-5(2H)-one (3), Figure 3 (a), shows methylene protons signal, 1, at \(\delta\) 1.67 while methylene protons of triazine ring, 2, appear downfield at \(\delta\) 2.21, this is due to the presence of (C=O) adjacent to it [30, 31]. The (N-H) singlet absorption occurs at \(\delta\) (4.77-5.88). These signals were further proved by D\(_2\)O exchange, Figure 3 (b). Furthermore, the \(^1\)H NMR spectrum of compound (4), Figure 4 (a), shows that the three methylene groups of diazepine ring appear as multiple signals in the region \(\delta\) (1.38-2.26). The other methylene group signal, 1, coincides with the signals of diazepine methylene groups. The (N-H) absorptions occur at \(\delta\) (4.77-5.44), which were further characterized by their disappearance due to D\(_2\)O exchange, aromatic protons appear at \(\delta\) (7.44-9.50), Figure 4 (b).
Figure 3. $^1$H NMR spectra of (a) 3-[(pyridine-2-ylamino)methyl]1,6-dihydro-1,2,4-triazine-5(2H)-one (3) without D$_2$O exchange; (b) with D$_2$O exchange
3.4. Carbon nuclear magnetic resonance ($^{13}$C NMR) analysis

Due to the scant solubility of the synthesized compounds, their spectra were recorded in d$_6$-DMSO. The $^{13}$C NMR spectrum of compound (3), Figure 5 (a), shows the following characteristic chemical shift. The two signals at δ 18.57 and δ 20.48 ppm are characteristic of the two methylene carbons, 1 and 2, respectively. The -C=C- absorption occurred at δ 52.48. Aromatic ring carbon absorption appears at δ (127.51-142.32). The signal at δ 173.32 is due to the absorption of the carbonyl carbon atom [32]. While, the $^{13}$C NMR spectrum of compound (4), Figure 5 (b), shows the following characteristic chemical shift. The signals at δ (16.23-20.05) are characteristic of the four methylene group carbons. Aromatic ring carbon atoms absorption appears at δ (127.25-132.02). The signals at δ (167.51-174.88) are due to the absorption of the three carbonyl carbon atoms [33]. These results give good support for the structure given to the synthesized compounds.
3.5. Antibacterial analysis

Antibacterial activities of the synthesized compounds against gram-negative (Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae) and gram-positive (Staphylococcus aureus, Bacillus cereus, and Enterococcus faecalis) bacterial strains were investigated using 5 mL of 100 μg/mL DMSO solutions of the tested compounds (1), (2), (3), and (4) in DMSO as a solvent and using two antibiotic drugs (Ceftriaxone, Amoxicillin) as references. The average diameter of the inhibition zones was measured, expressed in mm, and the minimum inhibitory concentration (MIC) is reported in μg/mL. The obtained results are shown in Table 2. All the tested compounds exhibited significant antibacterial activity. The compounds (3) and (4) showed the highest antimicrobial activity against all tested bacteria compared to compounds (1) and (2). The power of antibacterial activity of compounds (3) and (4) may be due to the existence of 1,2,4-triazine and 1,2-diazepine rings, which led to a significant increasing in antibacterial activity.

| Bacteria                        | Inhibition zone, mm (MIC, μg/mL) | Ceftriaxone | Amoxicillin |
|---------------------------------|----------------------------------|-------------|-------------|
|                                 |                                  | 1           | 2           | 3           | 4           |              |
| **Gram-negative**               |                                  |             |             |             |             |              |
| Klebsiella pneumoniae           | 9.9 (7.4)                        | 16.8 (15.2) | 26.4 (19.5) | 28.6 (22.7) | 32.2 (26.8) | 33.5 (25.4) |
| Escherichia coli                | 19.3 (14.2)                      | 21.1 (15.8) | 25.3 (22.8) | 27.3 (22.2) | 32.5 (25.6) | 35.6 (30.8) |
| Pseudomonas aeruginosa          | 13.6 (11.8)                      | 14.7 (10.3) | 30.1 (22.6) | 18.5 (13.8) | 33.1 (24.3) | 35.4 (28.7) |
| **Gram-positive**               |                                  |             |             |             |             |              |
| Bacillus cereus                 | 12.4 (10.7)                      | 10.4 (8.8)  | 28.5 (24.7) | 25.6 (20.1) | 34.4 (29.8) | 32.1 (26.3) |
| Enterococcus faecalis           | 13.5 (8.6)                       | 18.2 (10.1) | 22.5 (18.3) | 33.5 (26.2) | 36.7 (28.5) | 34.6 (29.9) |
| Staphylococcus aureus           | 15.1 (13.7)                      | 18.7 (12.5) | 22.6 (20.2) | 28.6 (21.7) | 30.1 (28.5) | 33.7 (29.7) |

4. Conclusions

In this study, we have provided an easy access for synthesis of novel 3-[(pyridine-2-ylamino)methyl]1,6-dihydro-1,2,4-triazine-5(2H)-one and 3-[(pyridine-2-ylamino)methyl]1,6-dihydro-1,2,4-triazine-5(2H)-one. The obtained results show significant antibacterial activity against both gram-negative and gram-positive bacterial strains.
yalamo)methyl][1,6-dihydro-1,2,4-triazine-5(2H)-one and 1-(N-pyridine-2-ylglycyl)-1,2-diazepine-3,7-dione. All spectroscopic methods used to analyze these compounds were in good agreement with the proposed structures. From the antimicrobial results, we found that these compounds exhibited promising antibacterial activity and can thus be considered potential antibacterial drugs. It can be concluded that those classes of compounds certainly hold great promise for discovering safer antibacterial agents.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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