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Synthesis and Antimicrobial Activity of Tetrazole-Substituted Compounds and Fused 1,3,4-Oxadiazoles

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Abstract

This study details the synthesis of novel tetrazole moieties fused with 1,3,4-oxadiazole rings. The structures of the synthesized compounds were confirmed through IR, ¹H-NMR, MS, and elemental analyses. Furthermore, the biological activities of the resulting compounds were evaluated against key bacterial strains, namely

Staphylococcus aureus, *Bacillus subtilis*, and *Escherichia coli*. The *in vitro* antifungal properties were also assessed using *Aspergillus niger* and *Candida albicans* fungal strains. This research provides a valuable framework for future investigations into the structure-activity relationships of these compounds.

Keywords: 5-amino-1H-tetrazole, 2-hydrazino-5-(5-amino-1H-tetrazol-1-yl) methyl-1,3,4-oxadiazole, Fused Heterocycles, Antimicrobial Activities

1. Introduction

Tetrazoles and their derivatives have garnered significant interest due to their diverse therapeutic and biological properties [1, 3]. They have demonstrated efficacy as antibacterial [4-17], antiproliferative [18], anticancer [18], and anticonvulsant agents [19]. The objective of this study is to expand the scope of research concerning the tetrazole scaffold and to assess its antimicrobial activities.

2. Materials and Methods

Melting points were measured using a Gallen Kamp apparatus and are uncorrected. Reactions were monitored, and product purification was performed *via* thin-layer chromatography (TLC) on precoated Silica Gel-Merck plates (0.25 mm layer thickness), with spot visualization using iodine. Infrared (IR) spectra were acquired in potassium bromide (KBr) on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded in DMSO-d₆ at 300 MHz on a Varian Mercury VX 300 NMR spectrometer, and chemical shifts (δ, ppm) are reported relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on an HP model MS 5988 spectrometer at an electron ionization energy of 70 eV. Microanalytical results were in satisfactory agreement with calculated values. The synthetic route for compound **4** is depicted in Scheme 1.

2.1 Methods

5-(5-Amino-1H-tetrazol-1-yl) methyl-2-hydrazino-1,3,4-oxadiazole (**5**, C₄H₇N₉O)

Compound **4** (5 mmol) was reacted with hydrazine hydrate (95%, 10 ml) in 15 ml ethanol under reflux for 2 h. The reaction mixture was allowed to cool, diluted with water, and acidified with hydrochloric acid. The resulting precipitate was collected by filtration, washed with water, and recrystallized from methanol, affording 0.82 g of the desired product (81.2% yield). Melting point: 244-246 °C. IR (selected bands): =3460, 3380 (NH₂), 3200 (NH), 1625 (C=N) cm⁻¹. ¹H NMR (DMSO-d₆): =11.25 (s, 1H, D₂O-exchangeable NH), 5.72 (s, 2H, D₂O-exchangeable NH₂), 5.50 (s, 2H, CH₂) ppm. MS: m/z (%) = 197 (M⁺, 22).

Synthesis of 5-(1-aminotetrazol-5-yl) methyl-2-arylidenehydrazino-1,3,4-oxadiazoles **6a-d** (General Procedure)

A solution 5 mmol of **5** in 15 ml methanol was added to 5 mmol of appropriate aromatic aldehyde and the mixture was heated at 100 °C for 10 min. The reaction mixture was kept at ambient temperature for overnight and the product which separated was

filtered off, washed with ether, dried, and crystallized from methanol. The physical-chemical and spectra data of **6a-d** the following:

5-(5-Amino-1H-tetrazol-1-yl) methyl-2-benzylidenehydrazino-1,3,4-oxadiazole (6a, C₁₁H₁₁N₉O)

The compound **6a** was obtained as a pale yellow solid in 81.1% yield (1.20 g). The melting point was determined to be 170-173 °C. Spectroscopic data are as follows: **IR** (selected peaks): 3325 cm⁻¹ (NH), 1625 cm⁻¹ (C=N); **¹H NMR** (DMSO-d₆): δ 11.60 (s, 1H, D₂O-exchangeable NH), 8.20-7.90 (m, 5H, Ar H), 7.61 (s, 1H, methylenic H), 5.72 (s, 2H, D₂O-exchangeable NH₂), 5.47 (s, 2H, CH₂) ppm; **MS**: m/z = 285 (M⁺, 13), 286 (M⁺+1, 18).

5-(5-Amino-1H-tetrazol-1-yl) methyl-2-p-tolylmethylidenehydrazino-1,3,4-oxadiazole (6b, C₁₂H₁₃N₉O)

The product **6b** was obtained as a yellow solid with a yield of 1.41g (88.1%) and a melting point of 150-152 °C. Spectroscopic data: **IR** (cm⁻¹): 3340 (NH), 1610 (C=N); **¹H NMR** (DMSO-d₆, ppm): 10.87 (s, 1H, D₂O-exchangeable NH), 8.17-7.82 (m, 4H, Ar H), 7.71 (s, 1H, methylenic H), 5.61 (s, 2H, D₂O-exchangeable NH₂), 5.54 (s, 2H, CH₂), 2.30 (s, 3H, CH₃); **MS**: m/z (%) = 299 (M⁺, 15).

5-(5-Amino-1H-tetrazol-1-yl) methyl-2-p-chlorobenzylidenehydrazino-1,3,4-oxadiazole (6c, C₁₁H₁₀ClN₉O)

The product **6c** was obtained as a yellow solid with a yield of 1.51g (88.8%) and a melting point of 174-176 °C. **IR** (cm⁻¹): 3350 (NH), 1610 (C=N). **MS**: m/z (%) = 320 (M⁺, 12).

5-(5-Amino-1H-tetrazol-1-yl) methyl-2-p-nitrobenzylidenehydrazino-1,3,4-oxadiazole (6d, C₁₁H₁₀N₁₀O₃)

The compound **6d** was obtained as an orange solid with a yield of 1.52 g (81.01%). It exhibits a melting point of 194-196 °C. **IR** spectroscopy revealed characteristic absorptions at 3340 cm⁻¹ (N-H stretching) and 1630 cm⁻¹ (C=N stretching). **¹H NMR** spectroscopy (DMSO-d₆) showed signals at δ 10.68 (s, 1H, D₂O-exchangeable, NH), 8.31-7.74 (m, 4H, Ar-H), 7.22 (s, 1H, methylenic H), 5.68 (s, 2H, D₂O-exchangeable, NH₂), and 5.58 (s, 2H, CH₂) ppm. **MS** displayed a molecular ion peak at m/z 330 (M⁺, 15%).

6-(1-Aminotetrazol-5-yl) methyl-3-aryl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazoles 7a-d (General procedure)

Method A. The respective hydrazone **7a-d** (5 mmol) was dissolved in 15 ml of glacial acetic acid. To this solution, a solution of bromine (5 mmol) in 10 ml of glacial acetic acid was added dropwise with continuous stirring. The resulting reaction mixture was then heated in a boiling water bath for 5 min, allowed to cool to room temperature, and subsequently poured into water. The precipitated solid was collected by filtration, washed extensively with water, and recrystallized from methanol.

Method B. The corresponding hydrazones **7a-d** (4 mmol) were dissolved in 20 ml of a 10% ethanolic ferric chloride solution and heated under reflux for 10 min. The resulting mixture was allowed to stand overnight at room temperature. The precipitated product was then collected by filtration, washed with water, dried, and recrystallized from methanol.

The aforementioned methods **A** and **B** are compatible with products **7a-d**, and their corresponding physical, chemical, and spectral data are as follows:

6-(5-Amino-1H-tetrazol-1-yl) methyl-3-phenyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazole (7a, C₁₁H₉N₉O)

The synthesis yielded the desired compound *via* two methods: Method **A** (0.61 g, 61.6%) and Method **B** (0.52 g, 52.5%). The product exhibited a melting point of 175-177 °C. Spectroscopic data included the following: **IR** (cm⁻¹): 3345 (NH), 1640 (C=N); **¹H NMR** (DMSO-d₆, ppm): 8.21-7.95 (m, 5H, ArH), 5.71 (s, 2H, D₂O-exchangeable NH₂), 5.54 (s, 2H, CH₂); **MS**: m/z (%) = 283 (M⁺, 15).

6-(5-Amino-1H-tetrazol-1-yl) methyl-3-p-tolyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazole (7b, C₁₂H₁₁N₉O)

The synthesis yielded the desired product **7b** with the following results: method **A**, 0.61 g (61.6%); method **B**, 0.81 g (81.8%). Melting point: 160-162 °C. **IR** (cm⁻¹): 3340 (NH), 1630 (C=N). **¹H NMR** (DMSO-d₆, ppm): 8.15-8.10 (m, 4H, Ar-H), 5.61 (s, 2H, D₂O-exchangeable NH₂), 5.56 (s, 2H, CH₂), 2.30 (s, 3H, CH₃). **MS**: m/z (%) = 298 (M⁺+1, 14).

6-(5-Amino-1H-tetrazol-1-yl) methyl-3-p-chlorophenyl-1,2,4-triazolo [3,4-b]-1,3,4-oxadiazole (7c, C₁₁H₈ClN₉O)

Yield: Method **A**, 0.55 g (55.5%); Method **B**, 0.52 g (52.5%). Melting point: 188-190 °C. **IR** (cm⁻¹): 3335 (NH), 1640 (C=N). **¹H NMR** (DMSO-d₆, ppm): 8.12-8.10 (m, 4H, Ar-H), 5.65 (s, 2H, D₂O-exchangeable NH₂), 5.52 (s, 2H, CH₂). **MS**: m/z (%) = 318 (M⁺, 20).

6-(5-Amino-1H-tetrazol-1-yl) methyl-3-p-nitrophenyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazole (7d, C₁₁H₈N₁₀O₃)

Methods **A** and **B** yielded 0.46g (46.5%) and 0.51g (51.5%) of the product **7d**, respectively. Melting point: 210-312 °C. **¹H NMR** (DMSO-d₆): δ 8.12-8.10 (m, 4H, Ar-H), 5.71 (s, 2H, D₂O-exchangeable NH₂), 5.54 (s, 2H, CH₂) ppm. **MS**: m/z (%) = 328 (M⁺, 20).

6-(5-Amino-1H-tetrazol-1-yl) methyl-3-methyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazole (8, C₆H₇N₉O)

Compound **8** (5 mmol) was refluxed in 10 ml of glacial acetic acid for 1 h. The mixture was then evaporated under reduced pressure, and the resulting residue was crystallized from methanol, yielding 0.92 g (81.4%) of the desired product; m.p. 220-222 °C; **IR** (selected bands): 3340 (NH), 1630 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): 5.62 (s, 2H, D₂O-exchangeable NH₂), 5.38 (s, 2H, CH₂), 2.42 (s, 3H, CH₃) ppm; **MS**: m/z (%) = 221 (M⁺, 20).

6-(5-Amino-1H-tetrazol-1-yl) methyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazol-3(2H)-one (9, C₅H₅N₉O₂)

The substrate (5 mmol) was suspended in 2 ml pyridine and treated with an excess of ethyl chloroformate. The resulting mixture was refluxed for 3 hours. Upon completion, the reaction mixture was poured into ice-water, which induced precipitation of the product **9**. The solid was collected *via* filtration, washed with water, and subsequently crystallized from methanol, yielding 0.85g (74.6%); m.p. 214 °C. Spectroscopic data: **IR** (cm⁻¹): 3300 (NH), 1690 (CON), 1625 (C=N); **¹H NMR** (DMSO-d₆, ppm): 11.85 (s, 1H, D₂O-exchangeable), 5.75 (s, 2H, D₂O-exchangeable NH₂), 5.40 (s, 2H, CH₂); **MS**: m/z (%) = 223 (M⁺, 22).

Synthesis of 10 and 11 (General Procedure)

To a solution of **5** (5 mmol) in 10 ml of methanol, 5 mmol of pyruvic acid or ethyl pyruvate was added. The mixture was maintained at ambient temperature for 24 h or heated at reflux for 1 h. The resulting precipitate was collected by filtration, washed with diethyl ether, and recrystallized from methanol to afford compounds **10** and **11**.

5-(5-Amino-1H-tetrazol-1-yl)-2-pyruvic acid hydrazino-1,3,4-oxadiazole (10, C₇H₉N₉O₃)

Yield: 0.92 g (66.7%); melting point: 178 °C; **IR** (cm⁻¹): 3450 (OH), 3225 (NH), 1715 (C=O), 1625 (C=N); **¹H NMR** (DMSO-d₆, δ, ppm): 12.52 (s, 1H, D₂O-exchangeable OH), 11.84 (s, 1H, D₂O-exchangeable NH), 5.72 (s, 2H, D₂O-exchangeable NH₂), 5.35 (s, 2H, CH₂), 2.50 (s, 3H, CH₃); **MS** (m/z (%)): 267 (M⁺, 27).

5-(5-Amino-1H-tetrazol-1-yl)-2-ethyl pyruvate hydrazino-1,3,4-oxadiazole (11, C₉H₁₃N₉O₃)

Yield: 1.21 g (78.6%); Melting point: 187 °C; **IR** (cm⁻¹): 3210 (NH), 1730 (C=O), 1600 (C=N); **¹H NMR** (DMSO-d₆, ppm): 11.61 (s, 1H, D₂O-exchangeable NH), 5.85 (s, 2H, D₂O-exchangeable NH₂), 5.29 (s, 2H, CH₂), 3.85 (q, 2H, CH₂CH₃), 2.55 (s, 3H, CH₃), 1.35 (t, 3H, CH₂CH₃); **MS**: m/z (%) = 295 (M⁺, 20).

7-(5-Amino-1H-tetrazol-1-yl)-3-methyl-1,3,4-oxadiazolo[2,3-c]-1,2,4-triazin-4(3H)-one (12, C₇H₇N₉O₂)

Compound **10** or **11** (5 mmol) was mixed with 10 ml of acetic acid and heated under reflux for 2 h. The resulting solution was then evaporated to dryness, and the residue was crystallized from methanol, yielding 0.61 g (65.6%) of the desired product **12**. The product exhibited a melting point of 222 °C. Spectroscopic data included the following: **IR** (=1690 (CON), 1620 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): =5.90 (s, 2H, D₂O-exchangeable NH₂), 5.34 (s, 2H, CH₂), 2.60 (s, 3H, CH₃) ppm; **MS**: m/z (%) = 249 (M⁺, 23).

7-(5-Amino-1H-tetrazol-1-yl)-methethyl-1-3-4-oxadiazolo[2,3-c]-1,2,4-triazin-3,4(2H)-dione (13, C₆H₅N₉O₃)

Compound **5** (5 mmol) and diethyl oxalate (5 mmol) were heated under reflux for 1 hour after reaching room temperature. The resulting mixture was triturated with methanol, and the precipitated product **13** was collected by filtration and crystallized from methanol, yielding 0.93g (71.8%). The product exhibited a melting point of 230 °C. Spectroscopic data included the following: **IR** (cm⁻¹): 3325 (NH), 1690, 1660 (CON), 1590 (C=N); **¹H NMR** (DMSO-d₆): δ 12.10 (s, 1H, D₂O-exchangeable NH), 5.75 (s, 2H, D₂O-exchangeable NH₂), 5.50 (s, 2H, CH₂) ppm; **MS**: m/z (%) = 251 (M⁺, 12).

Synthesis of 14 and 15 (General Procedure)

The reaction mixture, consisting of 5 mmol of the starting material **5** dissolved in 15 ml of methanol, was treated with 5 mmol of either acetylacetone or ethyl acetoacetate. The resulting mixture was then heated under reflux for 2 hours. Following the reaction, the precipitated product was isolated by filtration, washed with diethyl ether, and subsequently recrystallized from methanol to yield compounds **14** and **15**.

5-(5-Amino-1H-tetrazol-1-yl)-methyl-2-acetylacetonehydrazino-1,3,4-oxadiazole (14, C₉H₁₃N₉O₂)
Yield: 1.13 g (81.9%); m.p. 180 °C; **IR** (cm⁻¹): 3240 (NH), 1700 (C=O), 1625 (C=N); **¹H NMR** (DMSO-d₆, ppm): 11.85 (s, 1H, D₂O-exchangeable NH), 5.80 (s, 2H, D₂O-exchangeable NH₂), 5.40 (s, 2H, CH₂), 4.12 (s, 2H, CH₂), 2.35, 2.20 (2s, 3H each, 2CH₃); **MS**: m/z (%) = 279 (M⁺, 15).

5-(5-Amino-1H-tetrazol-1-yl)-methyl-2-ethyl acetoacetatehydrazino-1,3,4-oxadiazole (15, C₁₀H₁₅N₉O₃)
Yield: 1.22 g (78.7%); m.p. 192-194 °C; **IR** (cm⁻¹): 3295 (NH), 1735 (C=O), 1615 (C=N); **¹H NMR** (DMSO-d₆, ppm): 12.15 (s, 1H, D₂O-exchangeable NH), 5.78 (s, 2H, D₂O-exchangeable NH₂), 5.38 (s, 2H, CH₂), 4.20 (q, 2H, CH₂CH₃), 4.10 (s, 2H, CH₂), 2.40 (s, 3H, CH₃), 1.28 (t, 3H, CH₂CH₃); **MS**: m/z (%) = 310 (M⁺+1, 12).

5-(5-Amino-1H-tetrazol-1-yl)-2-(3,5-dimethylpyrazol-1-yl)-1,3,4-oxadiazole (16, C₉H₁₁N₉O)

Compound **14** (5 mmol) was dissolved in 10 ml of glacial acetic acid and heated under reflux for 2 h. The solvent was then removed under reduced pressure, and the resulting residue was crystallized from methanol to afford the product as a white solid. Yield: 0.53 g (57.0%); m.p. 195-197 °C; **IR** (selected): =1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): =5.75 (s, 2H, D₂O-exchangeable NH₂), 5.32 (s, 2H, CH₂), 5.25 (s, 1H, pyrazolyl CH), 2.30, 2.25 (2s, 3H each, 2CH₃) ppm; **MS**: m/z (%) = 261 (M⁺, 10).

5-(5-Amino-1H-tetrazol-1-yl)-2-(3-hydroxy-5-methylpyrazol-1-yl)-1,3,4-oxadiazole (17, C₈H₉N₉O₂)

The compound **15** (3 mmol) was dissolved in 15 ml of freshly prepared 0.1M sodium ethoxide solution and heated under reflux for 2 hours. The resulting solution was neutralized with acetic acid, and the precipitated product was collected by filtration and recrystallized from methanol. Yield: 0.54g (64.3%); m.p. 237-239 °C; **IR** (cm⁻¹): 3400 (OH), 1615 (C=N); **¹H NMR** (DMSO-d₆, ppm): 12.35 (s, 1H, D₂O-exchangeable OH), 5.80 (s, 2H, D₂O-exchangeable NH₂), 5.32 (s, 2H, CH₂), 5.30 (s, 1H, pyrazolyl CH), 2.35 (s, 3H, CH₃); **MS**: m/z (%) = 263 (M⁺, 30).

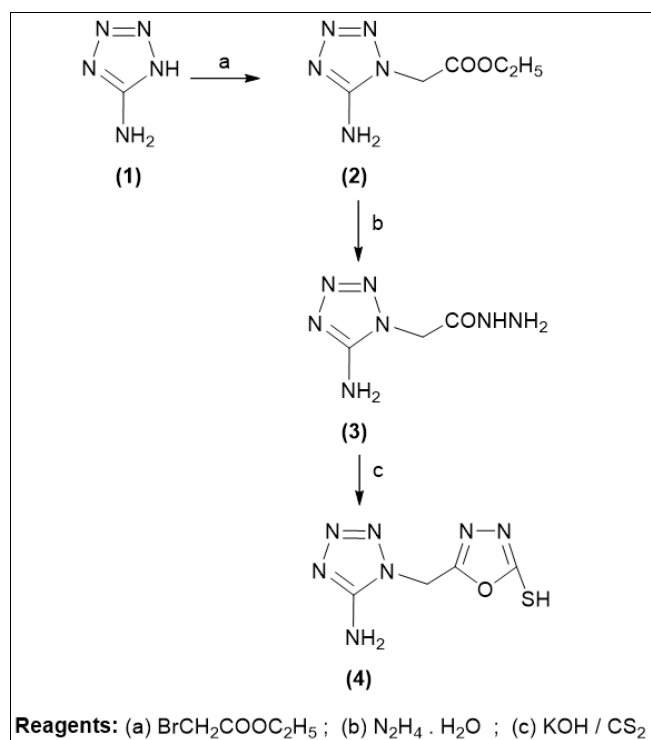
2.2 Biological Screening

The *in vitro* antimicrobial activity of the synthesized compounds was evaluated against a panel of bacteria. Disc diffusion assays [20, 21] were performed to assess activity against both *Gram*-positive and *Gram*-negative bacteria, which were dissolved in DMF at a concentration of 1000 ppm. Agar plates were uniformly inoculated with fresh broth cultures of the respective *Gram* bacteria. Discs were pre-incubated at 25 °C for 1 hour to facilitate diffusion, followed by incubation at 28 °C for 24 hours. Zones of inhibition were then measured.

3. Results and Discussion**3.1 Chemistry**

The reaction of 5-amino-1H-tetrazole (**1**) with ethyl bromoacetate produced ethyl 2-(5-amino-1H-tetrazol-1-yl)acetate (**2**). Subsequent treatment of compound **2** with hydrazine hydrate resulted in the direct formation of 2-(5-

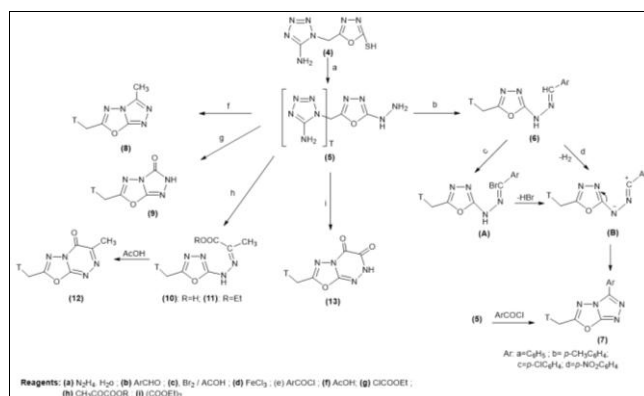
amino-1*H*-tetrazol-1-yl)acetohydrazide (3). Refluxing compound 3 with CS₂ in the presence of KOH yielded 5-(5-amino-1*H*-tetrazol-1-yl)methyl-1,3,4-oxadiazole-2-thiol (4). The synthetic steps for compounds 2-4 are outlined in Scheme 1. Furthermore, the reaction of compound 4 with hydrazine hydrate afforded the corresponding 5-(5-amino-1*H*-tetrazol-1-yl)methyl-2-hydrazino-1,3,4-oxadiazole (5). Condensation reactions of hydrazide 5 with an equimolar amount of the appropriate aromatic aldehyde in boiling methanol yielded the corresponding 5-(5-amino-1*H*-tetrazol-1-yl)methyl-2-arylidenehydrazino-1,3,4-oxadiazoles 6a-d, which exhibited characteristic IR absorptions for NH and NH₂, as well as ¹H NMR signals indicative of NH, NH₂ (D₂O-exchangeable), methylenic (CH=N), CH₂, and aromatic protons. Mass spectrometry revealed the correct molecular ions, which were further supported by elemental analyses. Dehydrogenative cyclization of hydrazide derivatives 6a-d with bromine in acetic acid or ethanolic FeCl₃, presumably *via* unstable hydrazonyl bromide intermediates [A] and [B], resulted in the formation of the corresponding 3-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazoles 7a-d. The IR spectra of these structures lacked the NH absorption, and the ¹H NMR spectra lacked the methylenic proton CH=N signal. Products 7a-d were also obtained through a one-pot cyclization of compound 5 with aromatic acid chlorides, which were formed in-situ and concomitantly cyclized to structures 7a-d (Scheme 2). The aforementioned products 7a-d were confirmed to be identical in all respects (m.p., mixed m.p., TLC, and IR) to those obtained *via* the cyclization methods described earlier in this article.



Scheme 1: Synthesis of compounds 2-4

The reaction of hydrazine 5 with excess glacial acetic acid yielded 6-(5-amino-1*H*-tetrazol-1-yl)methyl-3-methyl-1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazole 8. Intermediates of this reaction were not isolated. Conversely, heating hydrazine 5 with excess ethyl chloroformate in pyridine afforded a product lacking both ester-carbonyl absorption and ethyl group signals in the ¹H NMR spectrum. The product

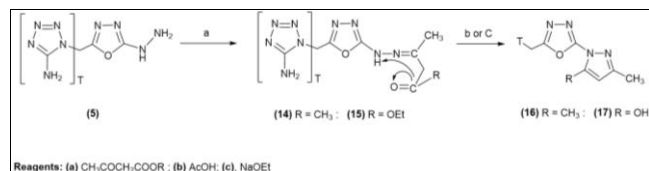
exhibited NH and CON absorptions, leading to its structural assignment as 6-(5-amino-1*H*-tetrazol-1-yl)methyl-1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazol-3(2*H*)-one (9). Subsequent condensation of 5 with pyruvic acid in boiling methanol resulted in the formation of the corresponding hydrazone 10, characterized by IR absorption bands indicative of OH, NH, and COOH groups. Similarly, reaction of ethyl pyruvate with hydrazine 5 yielded the hydrazone 11. The ¹H NMR spectrum of compound 11 displayed the characteristic triplet and quartet signal patterns associated with the ethyl group. Acid-catalyzed heterocyclization of either 10 or 11 *via* heating in acetic acid resulted in a single product. The IR spectrum of this product showed the disappearance of OH and NH absorptions, but retained a CON absorption. The ¹H NMR spectrum revealed the absence of the ethyl group pattern. These spectroscopic data, in conjunction with elemental analysis, support the structure of 7-(5-amino-1*H*-tetrazol-1-yl)methyl-3-methyl-1,3,4-oxadiazolo[2,3-*c*]-1,2,4-triazin-4(3*H*)-one (12). Furthermore, condensative cyclization of 2-hydrazino-1,3,4-oxadiazole structure 5 with an equimolar quantity of diethyl oxalate afforded 7-(5-amino-1*H*-tetrazol-1-yl)methyl-1,3,4-oxadiazolo[2,3-*c*]-1,2,4-triazin-3,4(2*H*)-dione (13). The structural assignment, as opposed to a possible intermediate hydrazido structure, was confirmed by elemental analysis and the absence of the triplet-quartet ¹H NMR signals characteristic of an ethyl group. The synthetic scheme for compounds 5-13 is depicted in Scheme 2.



Scheme 2: Synthesis of Compounds 5-13

The condensation of hydrazine 5 with acetylacetone afforded the corresponding hydrazone derivative 14, which exhibited characteristic IR absorptions for NH and C=O functionalities. The ¹H NMR spectrum of this product displayed signals corresponding to NH (D₂O-exchangeable), methylene, and methyl groups. Heating 14 with acetic acid induced cyclization to yield 5-(5-amino-1*H*-tetrazol-1-yl)methyl-2-(3,5-dimethylpyrazol-1-yl)-1,3,4-oxadiazole (16). The IR spectrum of 16 showed only C=N absorption, with the absence of NH and C=O absorptions characteristic of the parent hydrazone. Furthermore, the ¹H NMR spectrum exhibited a pyrazolyl CH proton signal. Similarly, condensation of ethyl acetoacetate with hydrazine 5 resulted in the formation of the hydrazone derivative 15, which underwent base-catalyzed cyclization upon heating with sodium ethoxide to furnish either the 1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazole 8 or the pyrazolyl derivative 17 through the elimination of ethyl acetate or ethanol. Evidence supporting the cyclization of hydrazone 15 includes: (i) the melting point and thin-layer chromatography behavior of the

obtained cyclization product, which differed from those expected for structure **8**, and (ii) spectroscopic data of this product, revealing OH absorption and the absence of amide absorption bands in the IR region; the ^1H NMR spectrum exhibited OH (D_2O -exchangeable) and pyrazolyl CH proton signals. Consequently, the product was definitively identified as 5-(5-amino-1*H*-tetrazol-1-yl)methyl-2-(3-hydroxy-5-methylpyrazol-1-yl)-1,3,4-oxadiazole (**17**). The synthetic route for compounds **14-17** is depicted in **Scheme 3**.



Scheme 3: Synthesis of Compounds **14-17**

3.2 Antimicrobial activity

The antimicrobial efficacy of the novel compounds **5,6a-d**, **7a-d**, and **8-17** was assessed.

The following bacterial and fungal strains were used in this study: *Gram-positive bacteria Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. Subtilis*); *Gram-negative bacteria Escherichia Coli* (*E. Coli*); and fungal strains *Aspergillums niger* (*A. niger*) and *Candida albicans* (*C. abicans*). The minimal inhibitory concentrations (MICs/mg/mL) are presented in **Table 1**.

Compounds **7a**, **7b**, **14**, and **15** demonstrated antimicrobial activity against *S. aureus* (25%). Compounds **8** and **9** exhibited activity against *B. subtilis* (25%). Compounds **10**, **11**, **15**, and **17** showed activity against *E. coli* (50%), comparable to that of ampicillin. Additionally, compounds **6b**, **8**, and **14** possessed antimycotic activity against *A. niger* (50%), and compounds **6a**, **8**, **14**, and **15** showed activity against *C. albicans* (50%), comparable to that of clotrimazole.

Table 1: Antimicrobial activity of synthesized compounds

Compound	Minimum Inhibitory Concentration (MIC) Expressed in mg/ml				
	Bacterial Strains			Fungal Strains	
	<i>S. aureus</i> (+)	<i>B. subtilis</i> (+)	<i>E. coli</i> (-)	<i>A. niger</i>	<i>C. albicans</i>
5	100	100	100	100	100
6a	100	100	>200	100	100
6b	>200	100	100	25	100
6c	100	100	100	100	100
6d	100	100	>200	100	25
7a	50	>200	100	100	100
7b	50	100	100	100	100
7c	>200	100	100	100	100
7d	100	100	100	100	100
8	100	50	>200	25	25
9	>200	50	100	100	100
10	100	100	50	100	100
11	100	100	50	25	100
12	100	>200	>200	100	100
13	100	>200	100	100	100
14	50	100	>200	25	25
15	50	100	50	100	25
16	100	100	100	100	100
17	100	100	50	100	100
Ampicillin	12.5	12.5	25	-	-
Clotrimazole	-	-	-	12.5	12.5

4. Conclusion

In summary, the present study highlights the effectiveness of 5-(5-amino-1*H*-tetrazol-1-yl) methyl-2-hydrazino-1,3,4-oxadiazole as a versatile synthon for synthesizing diverse condensed heterocyclic nitrogen structures through reactions with various cyclization reagents. Notably, the synthesized compounds exhibited antibacterial and antifungal activities that were comparable to those of ampicillin and clotrimazole, respectively.

5. Conflict of Interest Statement

There is no conflict of interest.

6. References

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