Int. j. adv. multidisc. res. stud. 2025; 5(5):1475-1480

Received: 05-09-2025
Accepted: 15-10-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

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

Mamdouh AM Taha
Department of Chemistry, Faculty of Science, Fayoum University, Fayoum 63514, Egypt

Corresponding Author: **Mamdouh AM Taha**

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 (^{1}H NMR) spectra were recorded in DMSO-d_6 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, $C_4H_7N_9O$)**
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 ºC. **IR** (selected bands): =3460, 3380 (NH_2), 3200 (NH), 1625 (C=N) cm^{-1}. **^{1}H NMR** (DMSO-d_6): =11.25 (s, 1H, D_2O-exchangeable NH), 5.72 (s, 2H, D_2O-exchangeable NH_2), 5.50 (s, 2H, CH_2) ppm. **MS**: m/z (%) = 197 (M^+, 22).

Synthesis of 5-(1-aminotetrazol-5-yl) methyl-2-arylidenehydrazino-1,3,4-oxadiazoles* 6_{a-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 ºC 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) mehyl-2-benzylidenehydrazino-1,3,4-oxadiazole* (6a, $C_{11}H_{11}N_9O$)**

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); **^{1}H NMR** (DMSO-d_6): δ 11.60 (s, 1H, D_2O-exchangeable NH), 8.20-7.90 (m, 5H, Ar H), 7.61 (s, 1H, methylenic H), 5.72 (s, 2H, D_2O-exchangeable NH_2), 5.47 (s, 2H, CH_2) 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, $C_{12}H_{13}N_9O$)**

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); **^{1}H NMR** (DMSO-d_6, ppm): 10.87 (s, 1H, D_2O-exchangeable NH), 8.17-7.82 (m, 4H, Ar H), 7.71 (s, 1H, methylenic H), 5.61 (s, 2H, D_2O- exchangeable NH_2), 5.54 (s, 2H, CH_2), 2.30 (s, 3H, CH_3); **MS**: m/z (%) = 299 (M^+, 15).

***5-(5-Amino-1H-tetrazol-1-yl) methyl-2-p-chlorobenzylienehydrazino-1,3,4-oxadiazole* (6c, $C_{11}H_{10}ClN_9O$)**

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, $C_{11}H_{10}N_{10}O_3$)**

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). **^{1}H NMR** spectroscopy (DMSO-d_6) showed signals at δ 10.68 (s, 1H, D_2O-exchangeable, NH), 8.31-7.74 (m, 4H, Ar-H), 7.22 (s, 1H, methylenic H), 5.68 (s, 2H, D_2O-exchangeable, NH_2), and 5.58 (s, 2H, CH_2) 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, $C_{11}H_9N_9O$)**

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); **^{1}H NMR** (DMSO-d_6, ppm): 8.21-7.95 (m, 5H, ArH), 5.71 (s, 2H, D_2O-exchangeable NH_2), 5.54 (s, 2H, CH_2); **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, $C_{12}H_{11}N_9O$)**

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). **^{1}H NMR** (DMSO-d_6, ppm): 8.15-8.10 (m, 4H, Ar-H), 5.61 (s, 2H, D_2O-exchangeable NH_2), 5.56 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). **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, $C_{11}H_8ClN_9O$)**

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). **^{1}H NMR** (DMSO-d_6, ppm): 8.12-8.10 (m, 4H, Ar-H), 5.65 (s, 2H, D_2O-exchangeable NH_2), 5.52 (s, 2H, CH_2). **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, $C_{11}H_8N_{10}O_3$)**

Methods **A** and **B** yielded 0.46g (46.5%) and 0.51g (51.5%) of the product **7d**, respectively. Melting point: 210-312 °C. **^{1}H NMR** (DMSO-d_6): δ 8.12-8.10 (m, 4H, Ar-H), 5.71 (s, 2H, D_2O-exchangeable NH_2), 5.54 (s, 2H, CH_2) 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, $C_6H_7N_9O$)**

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}; **^{1}H NMR** (DMSO-d_6): 5.62 (s, 2H, D_2O-exchangeable NH_2), 5.38 (s, 2H, CH_2), 2.42 (s, 3H, CH_3) 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, $C_5H_5N_9O_2$)**

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); **^{1}H NMR** (DMSO-d_6, ppm): 11.85 (s, 1H, D_2O-exchangeable), 5.75 (s, 2H, D_2O-exchangeable NH_2), 5.40 (s, 2H, CH_2); **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, $C_7H_9N_9O_3$)**
Yield: 0.92 g (66.7%); melting point: 178 °C; **IR** (cm^{-1}): 3450 (OH), 3225 (NH), 1715 (C=O), 1625 (C=N); **^{1}H NMR** (DMSO-d_6, δ, ppm): 12.52 (s, 1H, D_2O-exchangeable OH), 11.84 (s, 1H, D_2O-exchangeable NH), 5.72 (s, 2H, D_2O-exchangeable NH_2), 5.35 (s, 2H, CH_2), 2.50 (s, 3H, CH_3); **MS** (m/z (%)): 267 (M^+, 27).

***5-(5-Amino-1H-tetrazol-1-yl)-2-ethyl pyruvate hydrazino-1,3,4-oxadiazole* (11, $C_9H_{13}N_9O_3$)**
Yield: 1.21 g (78.6%); Melting point: 187 °C; **IR** (cm^{-1}): 3210 (NH), 1730 (C=O), 1600 (C=N); **^{1}H NMR** (DMSO-d_6, ppm): 11.61 (s, 1H, D_2O-exchangeable NH), 5.85 (s, 2H, D_2O-exchangeable NH_2), 5.29 (s, 2H, CH_2), 3.85 (q, 2H, CH_2CH_3), 2.55 (s, 3H, CH_3), 1.35 (t, 3H, CH_2CH_3); **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, $C_7H_7N_9O_2$)**
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}; **^{1}H NMR** (DMSO-d_6): =5.90 (s, 2H, D_2O-exchangeable NH_2), 5.34 (s, 2H, CH_2), 2.60 (s, 3H, CH_3) ppm; **MS**: m/z (%) = 249 (M^+, 23).

***7-(5-Amino-1H-tetrazol-1-yl) metheyl-1-3-4-oxadiazolo[2,3-c]-1,2,4-triazin-3,4(2H)-dione* (13, $C_6H_5N_9O_3$)**
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); **^{1}H NMR** (DMSO-d_6): δ 12.10 (s, 1H, D_2O-exchangeable NH), 5.75 (s, 2H, D_2O-exchangeable NH_2), 5.50 (s, 2H, CH_2) 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, $C_9H_{13}N_9O_2$)**
Yield: 1.13 g (81.9%); m.p. 180 °C; **IR** (cm^{-1}): 3240 (NH), 1700 (C=O), 1625 (C=N); **^{1}H NMR** (DMSO-d_6, ppm): 11.85 (s, 1H, D_2O-exchangeable NH), 5.80 (s, 2H, D_2O-exchangeable NH_2), 5.40 (s, 2H, CH_2), 4.12 (s, 2H, CH_2), 2.35, 2.20 (2s, 3H each, $2CH_3$); **MS**: m/z (%) = 279 (M^+, 15).

***5-(5-Amino-1H-tetrazol-1-yl) methyl-2-ethyl acetoacetatehydrazino-1,3,4-oxadiazole* (15, $C_{10}H_{15}N_9O_3$)**
Yield: 1.22 g (78.7%); m.p. 192-194 °C; **IR** (cm^{-1}): 3295 (NH), 1735 (C=O), 1615 (C=N); **^{1}H NMR** (DMSO-d_6, ppm): 12.15 (s, 1H, D_2O-exchangeable NH), 5.78 (s, 2H, D_2O-exchangeable NH_2), 5.38 (s, 2H, CH_2), 4.20 (q, 2H, CH_2CH_3), 4.10 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), 1.28 (t, 3H, CH_2CH_3); **MS**: m/z (%) = 310 (M^++1, 12).

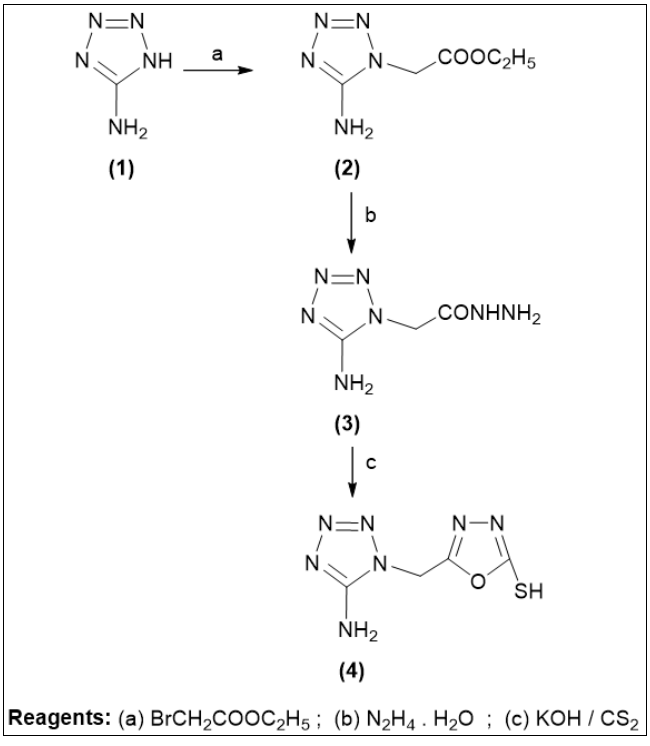
***5-(5-Amino-1H-tetrazol-1-yl)-2-(3,5-dimethylpyrazol-1-yl)1,3,4-oxadiazole* (16, $C_9H_{11}N_9O$)**
Compound **14** (5 mmol) was dissolved in 10 ml of glacial acetic acid and heated under reflux for 2 h. The solvent was then removed under reduced pressure, and the resulting residue was crystallized 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}; **^{1}H NMR** (DMSO-d_6): =5.75 (s, 2H, D_2O-exchangeable NH_2), 5.32 (s, 2H, CH_2), 5.25 (s, 1H, pyrazolyl CH), 2.30, 2.25 (2s, 3H each, $2CH_3$) ppm; **MS**: m/z (%) = 261 (M^+, 10).

***5-(5-Amino-1H-tetrazol-1-yl)-2-(3-hydroxy-5-methylpyrazol-1yl)-1,3,4-oxadiazole* (17, $C_8H_9N_9O_2$)**
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); **^{1}H NMR** (DMSO-d_6, ppm): 12.35 (s, 1H, D_2O-exchangeable OH), 5.80 (s, 2H, D_2O-exchangeable NH_2), 5.32 (s, 2H, CH_2), 5.30 (s, 1H, pyrazolyl CH), 2.35 (s, 3H, CH_3); **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.

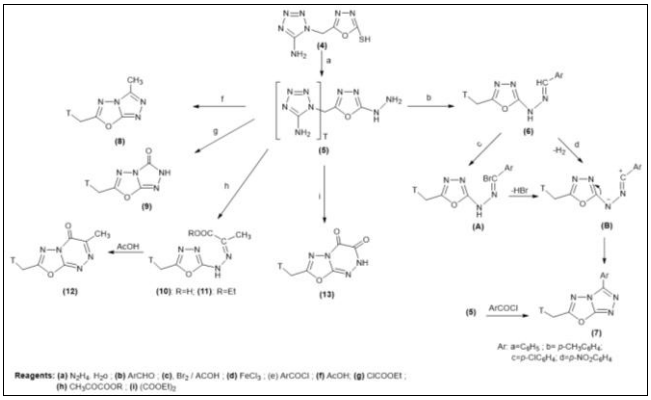
3. Results and Discussion
3.1 Chemistry
The reaction of 5-amino-1*H*-tetrazole (**1**) with ethyl bromoacetate produced ethyl 2-(5-amino-1*H*-tetrazol-1-yl)acetate (**2**). Subsequent treatment of compound **2** with hydrazine hydrate resulted in the direct formation of 2-(5-

amino-1*H*-tetrazol-1-yl)acetohydrazide (**3**). Refluxing compound **3** with CS_2 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 **6**$_{a-d}$, which exhibited characteristic IR absorptions for NH and NH_2, as well as ^{1}H NMR signals indicative of NH, NH_2 (D_2O-exchangeable), methylenic (CH=N), CH_2, and aromatic protons. Mass spectrometry revealed the correct molecular ions, which were further supported by elemental analyses. Dehydrogenative cyclization of hydrazide derivatives **6**$_{a-d}$ with bromine in acetic acid or ethanolic $FeCl_3$, 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 **7**$_{a-d}$. The IR spectra of these structures lacked the NH absorption, and the ^{1}H NMR spectra lacked the methylenic proton CH=N signal. Products **7**$_{a-d}$ 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 **7**$_{a-d}$ (**Scheme 2**). The aforementioned products **7**$_{a-d}$ 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**

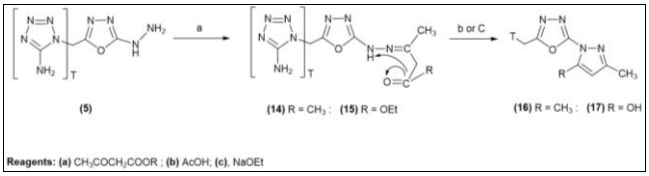
The reaction of hydrazine **5** with excess glacial acetic acid yielded 6-(5-amino-1*H*-tetrazol-1-yl)methyl-3-methyl-1,2,4-triazolo[3,4-b]-1,3,4-oxadiazole **8**. Intermediates of this reaction were not isolated. Conversely, heating hydrazine **5** with excess ethyl chloroformate in pyridine afforded a product lacking both ester-carbonyl absorption and ethyl group signals in the ^{1}H 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 ^{1}H 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 ^{1}H 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 ^{1}H 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 ^{1}H NMR spectrum of this product displayed signals corresponding to NH (D_2O-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 ^{1}H 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 ^{1}H 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**,**6**$_{a-d}$, **7**$_{a-d}$, 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 7$_a$, 7$_b$, 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 6$_b$, 8, and 14 possessed antimycotic activity against *A. niger* (50%), and compounds 6$_d$, 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*	
	S. aureus (+)	*B. subtilis (+)*	*E. coli (-)*	*A. niger*	*C. albicans*
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

1. Özkan H, Dmirci BJ. Het. CHem. 2019; 56(9):2528. Doi: https://doi.org/10.1002/jhet.3647
2. Moderhack DJ. Prakt. Chem. 1998; 340:687. Doi: https://doi.org/10.1002/prac.19983400802
3. Kolodobskii GI, Ostrovskii VA, Popavskii VS. Chem. Heterocycl. Cpds. 1981; 17:965. Doi: https://doi.org/10.1007/BF00503523
4. Ali Abdul-Ghaffar AT, Taha M, El-Shamy I, Mourad A. JSRS. 2021; 38:233-242. Doi: https://dx.doi.org/10.21608/jsrs.2021.48596.1039
5. Taha MAM. Eur. Int. J. Sci. Tech. 2020; 9:16. https://eijst.org.uk/files/articles/9.7.2.16-25.pdf
6. Khalifa NM, Al-Omar MA, Taha MM. Russ. J. Gen. Chem. 2017; 87(12):2966-2969. Doi: https://doi.org/10.1134/S1070363217120398
7. (a) Gein VL, Zamaraeva TM, Dmitriev MV. Russ. J. Org. Chem. 2016; 52:558. Doi: https://doi.org/10.1134/S107042801604014X
(b) Golobokova TV, Vereshchagin LI, Ratovskii GV, Proidakov AG, Kizhnyaev VN. Ibid. Russ. J. Org. Chem. 2016; 52:1039. Doi: https://doi.org/10.1134/S1070428016070216
8. El Badry SM, Taha MAM. J. Korean Chem. Soc. 2014; 58:381. Doi: http://dx.doi.org/10.5012/jkcs.2011.55.6.97
9. El-Badry SM, Taha MAM. J. Korean. Chem. Soc. 2011; 55:974.
10. Taha MAM, El-Badry SM. J. Korean. Chem. Soc. 2010; 54:414. Doi: https://doi.org/10.5012/jkcs.2010.54.4.414
11. Taha MAM, El-Badry SM. Monatsh. Chem. 2008; 139:1261. Doi: https://doi.org/10.1007/s00706-008-0902-8
12. Taha MAM. Phosphorus, Sulphur, Silicon Relat. Elem. 2008; 183:2525. Doi: https://doi.org/10.1080/10426500801967773
13. Taha MAM. Monatsh. Chem. 2007; 138:505. Doi: https://doi.org/10.1007/s00706-007-0599-0
14. Taha MAM, El-Badry SM. Phosphorus, Sulfur, Silicon Relat Elem. 2007; 182:1011. Doi: https://doi.org/10.1080/10426500601090768
15. Taha MAM, El-Badry SM. J. Chin. Chem. Soc. 2006; 53:1181. Doi: https://doi.org/10.1002/jccs.200600156
16. Taha MAM. J. Chin. Chem. Soc. 2005; 52:137. Doi: https://doi.org/10.1002/jccs.200500021
17. Karnik AV, Malviya NJ, Kulkarni AM, Jadhav BL. Eur. J. Med. Chem. 2006; 41:891. Doi: https://doi.org/10.1016/j.ejmech.2006.01.018

18. Jantova S, Ruzekova I, Stantovsky S, Spirkova K. Neoplasma. 1997; 44:240. https://pubmed.ncbi.nlm.nih.gov/9473777/
19. Rubat C, Coudert P, Couquelet JM, Tronche P, Bastide J, Bastide P. Formaco. 1990; 45:331. Doi: https://doi.org/10.1248/cpb.37.2832
20. Colle GJ, Fraser GA, Marmion PB, Simmons A. Practical Medical Microbiology, 14th Edn., Churchill Livingstone: Edinburgh. 1996; 11:163. https://openlibrary.org/books/OL21216804M
21. Arthington BA, Motley M, Warmock DW, Morrison CJ. J. Clin. Microbiology. 2000; 38:2254. https://pmc.ncbi.nlm.nih.gov/articles/PMC85559/