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Synthesis, Characterization and Antimicrobial Evaluation of New 5-Acetamido-3-acetyl-1,3,4-oxadiazoline Derivatives

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Abstract

A new series of 5-acetamido-3-acetyl-1,3,4-oxadiazoline derivatives were synthesized via oxidative cyclization reaction of different N-carbamoylhydrazones with acetic anhydride. The structures of obtained compounds were confirmed by IR, MS, ^{1}H NMR, ^{13}C NMR and Elemental analysis methods and are in full agreement with their molecular structure. The synthesized 1,3,4-oxadiazolines were screened for in vitro for their biological activity against a variety of bacterial strains (*Enterococci, Escherichia coli, Staphylococcus aureus, Klebsiella spp, Proteus spp*), and fungi (*Aspergillus niger, Candida albicans*), employing the nutrient agar disc diffusion method. The obtained results showed that these compounds have good inhibition against the tested pathogens.

Keywords: N-acetyl-1,3,4-oxadiazolines; N-carbamoylhydrazones; Biological activity; Oxidative cyclization

Introduction

Nitrogen containing heterocycles play an important role in the drug discovery process. The nitrogenated frames commonly formed as fractions in the structure of most drugs with various ring sizes; aromatic and nonaromatic rings; fused and bicyclic rings. Nitrogen heterocyclic medications are present in all therapeutic areas including cardiovascular and metabolic illnesses, Central Nervous System (CNS) disorders, anti-inflammatory, antineoplastic, anti-infective drugs, and among others. In drug discovery and development, oxadiazole rings are commonly employed as bioisosteric replacement for carbonyl-containing groups such as esters, amides, carbamates and hydroxamic esters. Such groups are usually unstable in 2 biological media, which poses as hurdle for their use in the structure of drug candidates. Over the last years, bioactive molecules containing the oxadiazole ring have been increasingly discovered, developed and studied. Moreover, the oxadiazole chemistry has been developed extensively and is still developing continuously. Presently it is possible to find the oxadiazole moiety in various compounds possess bioactivities as antitumor, antitubercular, anticonvulsant, antioxidant, antibacterial, antiviral, anti-inflammatory, insecticidal, antiparasitic agents, and cardioprotective agents [1-3].

Since 1950 a diversified reactions, various precursors are used to obtain an oxadiazoles having novel pharmacological activity [4]. Among them the substituted-1,3,4-oxadiazole derivatives have attracted considerable attention owing to their effective biological activity and extensive use in medication industries [5,6].There are many reviews of the literature available on the correlation between the chemical structure and the biological activity of 1,3,4-oxadiazole derivatives [7]. Typically, several oxidizing agents were used to create 1,3,4-oxadiazole derivatives from acylhydrazones, as Lead Tetraacetate (LTA) in CH_2Cl_2, Phenyliodine (III) Diacetate (PIDA) in ethanol, electrolytic oxidation in methanolic sodium acetate ($AcONa/CH_3OH$) involving intramolecular cyclization to give 2-methoxy-1,3,4- oxadiazoline [3,8-12]. Recently Ultrasound-assisted synthesis of 2-amino-1,3,4-oxadiazoles through NBS-mediated oxidative cyclization of semicarbazones has been reported [13].

Using acid anhydride was the most common procedure of cyclization of acylhydrazones. The Acetic Anhydride (AA) is frequently

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used as dehydrant in these kinds of reactions in many literatures. The treatment of acylhydrazones with acetic anhydride found to give 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines with good yield [14-18]. Therefore, as a part of our program focused on 1,3,4-oxadiazole with biological activity, and in connection with our interest in the chemistry of 1,3,4-oxadiazole. We outlined in the present research, the synthesis of some novel 3-cetyl-5-acetamido-1,3,4-oxadiazolines and their antimicrobial activity were investigated.

Experimental

Chemicals and instrumentations

All chemicals and reagents used in this research were purchased from Sigma-Aldrich (Germany), Merck Co. (Germany), Fluka Chemie Company (Switzerland) and Acros company (Belgum), and used without further purification (unless otherwise stated) where the manufacturer declared their class of purity. The purity of the obtained compounds was assessed by means of Thin Layer Chromatography (TLC) on plates of silica gel (60 F-254) supplied by 3 Merck Co. The melting points of the obtained compounds were determined on open capillary tube using a Stuart melting point apparatus (England) equipped with a thermometer and presented without any correction. The IR spectra were recorded on a Nicolet 6700 spectrometer (Thermo Scientific, Madison, WI, USA); in cm^{-1}.

The ^{1}H NMR spectra were recorded in (DMSO-d6) at 300 MHz and ^{13}C NMR spectra at 75 MHz on a Bruker AM 300 NMR spectrometer. Chemical shift (δ) values are donated in ppm relative to Tetramethylsilane (TMS) as internal standard. The splitting patterns for NMR spectra are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or combinations thereof. Coupling constants (J) are designated in Hz. Electron impact mass spectra were run on Finnegan MAT 8200 and 8400 Mass spectrometers at 70 eV. The elemental analysis was carried out at Microanalysis center of Cairo University, Giza, Egypt, and the obtained compounds analyzed satisfactorily for C, H and N and the results were within ± 0.3% - 0.4% of the theoretical values.

Synthesis

General procedure for synthesis of carbamoyl hydrazones 3a-s: A mixture of semicarbazide hydrochloride 1 (15 mmol), aldehydes or ketones 2a-s (15 mmol), sodium acetate (15 mmol) and few drops of glacial acetic acid in methanol (30 mL) was stirred under reflux until reaction had completed (1 hr-2 hrs.). The reaction mixture was allowed to cool to room temperature, and the solid precipitate was filtered and recrystallized from ethanol or methanol to give the desired semicarbazones 3a-s in 85%-95% yield [13,19,20].

General procedure for synthesis of 1,3,4-oxadiazolines 4a-s: A mixture of carbamoyl hydrazones 3a-s (15 mmol-20 mmol) and excessive acetic anhydride (15 mL-25 mL) was stirred under reflux in oil bath for 2 hrs-3 hrs. After the reaction was completed (controlled by TLC), the reaction mixture was allowed to cool and the formed precipitate was collected and washed with potassium carbonate solution (10%) followed by water and dried. In some cases, where the precipitate not formed on cooling, the reaction mixture left for 2-3 days to vaporize the excess anhydride or removed invacuo, and the residue washed with potassium bicarbonate solution (10%) and finally with water and dried. In all cases the resulting crude solid product was recrystallized from ethanol or ethyl acetate and air-dried to afford 1,3,4- oxadiazolines 4a-s.

5-Acetamido-3-acetyl-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4a): White solid (78% yield), m.p. 182°C-184°C. FTIR (KBr, cm^{-1}): 3267 (NH), 1675, 1670 (2C=O), 1610 (C=N), 1159 (C-OC). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.62 (d, J = 5.0 Hz, 3H, CH_3 of C_2-oxad.), 2.32, 2.35 4 (s, 6H, $2CH_3$), 6.33 (q, J = 5.4 Hz, 1H, CH of C_2-oxad.), 11.35 (s, 1H, NH). ^{13}C NMR (DMSOd6, 75 MHz): δ = 23.6, 23.3 ($2CH_3$), 24.6 (CH_3), 91.6 (C_2-oxad.), 150.3 (C=N, oxadiazole ring), 167.2, 166.9 (2C=O). MS: m/z 185 [$M^{+\cdot}$]; Anal. Cald. for $C_7H_{11}N_3O_3$: C, 45.40; H, 5.99; N, 22.69%. Found; C, 45.18; H 6.16; N, 22.51%.

5-Acetamido-3-acetyl-2-ethyl-2,3-dihydro-1,3,4-oxadiazole (4b): White solid (75% yield), m.p. 169°C -172°C. FTIR (KBr, cm^{-1}): 3269 (NH), 1676, 1668 (2C=O), 1608 (C=N), 1163 (C-OC). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.05-1.11 (t, J = 7.0 Hz, 3H, CH_3 ethyl), 1.60-1.63 (m, 2H, CH_2 ethyl), 2.31-2.35 (s, 6H, $2CH_3$), 6.28 (t, J = 6.0 Hz, ^{1}H, CH at C_2-oxad.), 11.33 (s, ^{1}H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.3 (CH_3 ethyl), 18.7 (CH_2 ethyl), 23.5, 23.3 ($2CH_3$), 92.5 (C_2-oxad.), 149.6 (C=N, oxad.), 166.9, 167.4 (2C=O). MS: m/z 199 [M^{+}]; Anal. Cald. for $C_8H_{13}N_3O_3$: C, 48.23; H, 6.58; N, 21.09%. Found; C, 48.42; H 6.41; N, 20.92%.

5-Acetamido-3-acetyl-2-n-propyl-2,3-dihydro-1,3,4-oxadiazole (4c): White solid (77% yield), m.p. 176°C-178°C. FTIR (KBr, cm^{-1}): 3258 (NH), 1669, 1664 (2C=O), 1605 (C=N), 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.95-1.01 (t, 3H, CH_3), 1.58-1.66 (m, 2H, CH_2), 2.00-2.26 (t, 2H, CH_2), 2.33-2.36 (s, 6H, $2CH_3$), 6.30 (t, J = 6.0 Hz, 1H, CH at C_2-oxad.), 11.25 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 13.0 (CH_3), 16.9 (CH_2), 18.8 (CH_2), 23.5, 23.3 ($2CH_3$), 92.7 (C_2-oxad.), 149.6 (C=N, oxad.), 166.9/166.4 (2C=O).MS: m/z 213 [$M^{+\cdot}$]; Anal. Cald. for $C_9H_{15}N_3O_3$: C, 50.69; H, 7.09; N, 19.71%. Found; C, 50.91; H 6.91; N, 19.56%.

5-Acetamido-3-acetyl-2-n-hexyl-2,3-dihydro-1,3,4-oxadiazole (4d): White solid (73% yield), m.p. 187°C-189°C. FTIR (KBr, cm^{-1}): 3272 (NH), 1670, 1665 (2C=O), 1610 (C=N), 1154 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 0.65 (t, J = 6.0 Hz, 3H, hexyl group), 1.56-1.08 (m, 8H, $4CH_2$, hexyl protons), 1.86 (q, J = 6.0 Hz, 2H, hexyl group), 2.30-2.33 (s, 6H, $2CH_3$), 6.36 (t, J = 6.0 Hz, 1H, CH at C_2-oxad.), 11.26 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.7 ($2CH_3$), 26.7, 24.6, 19.6, 13.8, 11.7 ($5CH_2$), 7.6 (CH_3) (6 hexyl carbons), 92.4 (C_2-oxad.), 149.7 (C=N, oxad.), 166.8, 166.4 (2C=O). MS: m/z 255 [M^+]; Anal. Cald. for $C_{12}H_{21}N_3O_3$: C, 56.45; H, 8.29; N, 16.46%. Found; C, 56.75; H 8.44; N, 16.63%.

5-Acetamido-3-acetyl-2-cyclohexyl-2,3-dihydro-1,3,4-oxadiazole (4e): White solid (75% yield), m.p. 193°C -195°C. FTIR (KBr, cm^{-1}): 3264 (NH), 1670, 1666 (2C=O), 1611 (C=N), 1152 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.96-1.20 (m, 11H, cyclohexyl protons), 2.30-2.33 (s, 6H, $2CH_3$), 6.35 (d, J = 6.0 Hz, 1H, CH at C_2-oxad.), 11.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.7 ($2CH_3$), 40.3 (CH), 27.6 ($2CH_2$), 25.3 ($2CH_2$), 24.2 (CH_2) (cyclohexyl carbons), 92.6 (C_2-oxad.), 146.9 (C=N, oxad.), 166.9, 167.2 (2C=O).MS: m/z 253 [$M^{+\cdot}$]; Anal. Cald. for $C_{12}H_{19}N_3O_3$: C, 56.90; H, 7.56; N, 16.59%. Found; C, 56.63; H 7.73; N, 16.42%.

5-Acetamido-3-acetyl-2-phenyl-2,3-dihydro-1,3,4-oxadiazole (4f): White solid (68% yield), m.p. 179°C-181°C. FTIR (KBr, cm^{-1}): 3258 (NH), 3033 (Ar-H), 1676, 1667 (2C=O), 1598 (C=N), 1162 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.30-2.33 (s, 6H, $2CH_3$), 7.04 (s, 1H, CH at C_2-oxad.), 7.34-7.93 (m, 5H, arom.), 11.09 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.7 ($2CH_3$), 89.3 (C_2-oxad.), 134.2, 130.8, 128.7, 127.0, 126.7, 122.3 (6 arom. C), 149.8 (C=N, oxad.), 166.9, 167.3 (2C=O). MS: m/z 247 [$M^{+\cdot}$]; Anal. Cald. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99%. Found; C, 58.60; H 5.46; N, 17.16%.

5-Acetamido-3-acetyl-2-(4-chlorophenyl)-2,3-dihydro-1,3,4oxadiazole (4g): Yellow solid (70% yield), m.p. 166°C-169°C. FTIR (KBr, cm^{-1}): 3262 (NH), 2929, 2840 (Ar-H), 1678, 1668 (2C=O), 1604 (C=N), 1162 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.31-2.35 (s, 6H, $2CH_3$), 7.08 (s, 1H, CH at C_2-oxad.), 7.34-7.93 (m, 4H, arom.), 11.04 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.4 ($2CH_3$), 89.7 (C_2-oxad.), 133.2, 131.2, 128.1, 125.7, 123.3, 119.2 (6 arom. C), 150.3 (C=N, oxad.), 166.8, 167.2 (2C=O). MS: m/z 281/283 [$M^{+\cdot}$]; Anal. Cald. for $C_{12}H_{12}C_lN_3O_3$: C, 51.17; H, 4.29; N, 14.92%. Found; C, 50.89; H 4.46; N, 14.74%.

5-Acetamido-3-acetyl-2-(2-furyl)-2,3-dihydro-1,3,4-oxa-diazole (4h): White solid (72% yield), m.p. 167°C-169°C. FTIR (KBr, cm^{-1}): 3273 (NH), 2943 (Ar-H), 1667, 1657 (2C=O), 1593 (C=N), 1157 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.30-2.33 (s, 6H, $2CH_3$), 7.90 (dd, J = 1.8 Hz, 1H furan ring), 7.71 (dd, J = 3.5 Hz, 1H furan ring), 7.56 (d, J = 3.5 Hz, 1H furan ring), 7.03 (s, 1H, CH at C_2-oxad.), 11.05 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.2, 23.4 ($2CH_3$), 88.6 (C_2-oxad.), 133.7, 131.5, 128.4, 127.2 (4 arom. C), 153.9 (C=N, oxad.), 166.4, 166.9 (2C=O). MS: m/z 237 [$M^{+\cdot}$]; Anal. Cald. for $C_{10}H_{11}N_3O_4$: C, 50.63; H, 4.67; N, 17.71%. Found; C, 50.91; H 4.52; N, 17.58%.

5-Acetamido-3-acetyl-2-(2-thienyl)-2,3-dihydro-1,3,4-oxadiazole (4i): Light yellow (65% yield), m.p. 206°C-208°C. FTIR (KBr, cm^{-1}): 3257 (NH), 2953 (Ar-H), 1669, 1658 (2C=O), 1602 (C=N), 1159 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.33-2.38 (s, 6H, $2CH_3$), 7.82 (d, J = 1.3 Hz, 1H thiophene ring), 7.72 (d, J = 3.4 Hz, 1H thiophene ring), 7.64 (d, J = 3.4 Hz, 1H thiophene ring), 7.07 (s, 1H, CH at C_2-oxad.), 11.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.3, 23.5 ($2CH_3$), 87.9 (C_2-oxad.), 130.8, 129.9, 128.2, 125.3 (4 arom. C), 147.9 (C=N, oxad.), 166.3, 167.0 (2C=O). MS: m/z 253 [$M^{+\cdot}$]; Anal. Cald. for $C_{10}H_{11}N_3O_3S$: C, 47.42; H, 4.38; N, 16.59%. Found; C, 47.16; H 4.56; N, 16.76%.

5-Acetamido-3-acetyl-2,2-dimethyl-2,3-dihydro-1,3,4-oxadiazole (4j): Pale brown solid (86% yield), m.p. 198°C-201°C. FTIR (KBr, cm^{-1}): 3253 (NH), 1668, 1659 (2C=O), 1605 (C=N), 1165 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.53-1.56 (s, 6H, $2CH_3$ of C_2 oxad.), 2.26- 2.36 (s, 6H, $2CH_3$), 10.97 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.3, 23.6 ($2CH_3$), 6 24.3, 24.4 ($2CH_3$ at C_2-oxad.), 90.2 (quaternary C_2-oxad.), 147.6 (C=N, oxad.), 168.4, 169.9 (2C=O). MS: m/z 199 [$M^{+\cdot}$]; Anal. Cald. for $C_8H_{13}N_3O_3$: C, 48.23; H, 6.58; N, 21.09%. Found; C, 48.50; H 6.39; N, 20.91%.

5-Acetamido-3-acetyl-2-ethyl-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4k): White solid (82% yield), m.p. 178°C-180°C. FTIR (KBr, cm^{-1}): 3268 (NH), 1673, 1668 (2C=O), 1601 (C=N), 1156 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.04 (t, J = 7.5 Hz, 3H, CH_3 of ethyl), 1.64 (q, 2H, CH_2 of ethyl), 2.13 (s, 3H, CH_3 of C_2 oxad.), 2.33-2.38 (s, 6H, $2CH_3$), 10.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 9.6 (CH_3), 22.5 (CH_2), 23.3, 23.6 ($2COCH_3$), 24.6 (CH_3 at C_2-oxad.), 83.5 (C_2-oxad.), 154.5 (C=N, oxad.), 166.5, 167.6 (2C=O). MS: m/z 213[$M^{+\cdot}$]; Anal. Cald. for $C_9H_{15}N_3O_3$: C, 50.69; H, 7.09; N, 19.71%. Found; C, 50.38; H 7.30; N, 19.92%.

5-Acetamido-3-acetyl-2-cyclopropyl-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4l): Offwhite solid (76% yield), m.p. 217°C-219°C. FTIR (KBr, cm^{-1}): 3274 (NH), 1672, 1667 (2C=O), 1597 (C=N), 1249 (C-O-C). ^1H NMR (DMSO-d_6, 300 MHz): δ = 0.55-0.67 (m, 4H, $2CH_2$ of cyclopropyl), 1.26-1.32 (m, 1H, CH of cyclopropyl), 1.64 (s, 3H, CH_3 of C_2 oxad.), 2.38 (s, 6H, $2CH_3$), 11.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ =3.1, 3.2 ($2CH_2$ cyclopropyl), 9.9 (CH cyclopropyl), 23.2, 23.5 ($2COCH_3$), 24.3 (CH_3 at C_2-

oxad.), 83.4 (C_2-oxad.), 154.2 (C=N, oxad.), 166.8, 167.3 (2C=O). MS: m/z 225 [M^{+}]; Anal. Cald. for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N, 18.65%. Found; C, 53.59; H 6.52; N, 18.83%.

5-Acetamido-3-acetyl-2-methyl-2-phenyl-2,3-dihydro-1,3,4-oxadiazole (4m): White solid (72% yield), m.p. 196°C-198°C. FTIR (KBr, cm^{-1}): 3258 (NH), 3033, 2840 (Ar-H), 1656, 1648 (2C=O), 1611 (C=N), 1159 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.54 (s, 3H, CH_3 of C_2 oxad.), 2.27-2.33 (s, 6H, $2CH_3$), 7.34-7.93 (m, 4H, arom.), 11.04 (s, 1H, NH). ^{13}C NMR (DMSO-d6, 75 MHz): δ = 23.4 ($2CH_3$), 24.3 (CH_3 at C_2-oxad.), 89.7 (C_2-oxad.), 133.2, 131.2, 128.1, 127.6, 125.7, 121.6 (6 arom. C), 151.3 (C=N, oxad.), 166.8, 167.2 (2C=O).MS: m/z 261 [M^{+}]; Anal. Cald. for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08%. Found; C, 59.51; H 5.61; N, 15.91%

5-Acetamido-3-acetyl-2-(4-bromophenyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4n): Light yellow solid (80% yield), m.p. 180°C-182°C. FTIR (KBr, cm^{-1}): 3257 (NH), 2923, 2833 (Ar-H), 1660, 1652 (2C=O), 1597 (C=N), 1149 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.51 (s, 3H, CH_3 of C_2 oxad.), 2.27-2.33 (s, 6H, $2CH_3$), 7.56 (d, J = 4.2 Hz, 2H arom.), 7.34 (d, J = 2.1 Hz, 2H arom.), 11.00 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.6 (2CH3), 24.6 (CH_3 at C_2-oxad.), 83.7 (C_2-oxad.), 133.7, 130.9, 128.5, 126.9, 123.7, 114.6 (6 arom. C), 7 149.9 (C=N, oxad.), 166.5, 167.6 (2C=O). MS: m/z 340/342 [M^{+}]; Anal. Cald. for $C_{13}H_{14}BrN_3O_3$: C, 45.90; H, 4.15; N, 12.35%. Found; C, 46.18; H 4.33; N, 12.54%.

5-Acetamido-3-acetyl-2-(4-chlorophenyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4o): Pale yellow solid (68% isolated yield), m.p. 207°C-210°C. FTIR (KBr, cm^{-1}): 3253 (NH), 2925, 2841 (Ar-H), 1676, 1667 (2C=O), 1611 (C=N), 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.51 (s, 3H, CH_3 of C_2 oxad.), 2.28-2.35 (s, 6H, $2CH_3$), 7.41 (d, J = 4.1 Hz, 2H arom.), 7.31 (d, J = 2.0 Hz, 2H arom.), 11.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.6 ($2CH_3$), 24.6 (CH_3 at C_2-oxad.), 83.8 (C_2-oxad.), 130.7, 128.9, 127.6, 123.5, 122.9, 118.7 (6 arom. C), 150.5 (C=N, oxad.), 166.7, 167.2 (2C=O). MS: m/z 295/297 [M^{+}]; Anal. Cald. for $C_{13}H_{14}ClN_3O_3$: C, 52.80; H, 4.77; N, 14.21%. Found; C, 52.52; H 4.96; N, 14.01%.

5-Acetamido-3-acetyl-2-(2-furyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4p): Off-White solid (73% isolated yield), m.p. 175°C-177°C. FTIR (KBr, cm^{-1}): 3272 (NH), 2939 (Ar-H), 1672, 1665 (2C=O), 1613 (C=N), 1158 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.54 (s, 3H, CH_3 of C_2 oxad.), 2.26-2.32 (s, 6H, $2CH_3$), 7.97 (dd, J=1.8 Hz, 1H furan ring), 7.69 (dd, J = 3.5 Hz, 1H furan ring), 7.54 (d, J = 3.5 Hz, 1H furan ring), 11.06 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.4 ($2CH_3$), 24.3 (CH_3 at C_2-oxad.), 90.4 (C_2-oxad.), 130.3, 129.8, 128.2, 124.8 (4 C furan ring), 154.6 (C=N, oxad.), 167.0, 167.3 (2C=O). MS: m/z 251 [M^{+}]; Anal. Cald. for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.72%. Found; C, 52.87; H, 5.41; N, 16.53%.

5-Acetamido-3-acetyl-2-(2-thienyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4q): White solid (74% isolated yield), m.p. 164°C-166°C. FTIR (KBr, cm^{-1}): 3257 (NH), 2945 (Ar-H), 1673, 1667 (2C=O), 1609 (C=N), 1162 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.52 (s, 3H, CH_3 of C_2 oxad.), 2.24-2.30 (s, 6H, $2CH_3$), 7.92 (d, J = 1.3 Hz, 1H thiophene ring), 7.68 (d, J = 3.4 Hz, ^{1}H thiophene ring), 7.48 (d, J = 3.4 Hz, 1H thiophene ring), 11.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.6 ($2CH_3$), 24.4 (CH_3 at C_2-oxad.), 90.6 (C_2-oxad.), 131.8, 128.9, 127.0, 125.3 (4 arom. C), 153.9 (C=N, oxad.), 167.1, 167.3 (2C=O). MS: m/z 267 [M^{+}]; Anal. Cald. for $C_{11}H_{13}N_3O_3S$: C, 49.43; H, 4.90; N, 15.72%. Found; C, 49.71; H 4.09; N, 15.53%.

5-Acetamido-3-acetyl-2,2-dicyclopropyl-2,3-dihydro-1,3,4-oxadiazole (4r): White solid (66% isolated yield), m.p. 243°C dec. FTIR (KBr, cm^{-1}): 3255 (NH), 1672, 1660 (2C=O), 1612 (C=N), 1161 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.60-0.75 (m, 8H, $4CH_2$ of cyclopropyl), 1.29-1.34 (m, 2H, 2CH of cyclopropyl), 2.22-2.29 (s, 6H, $2CH_3$), 11.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 3.1, 3.2 ($4CH_2$ cyclopropyl), 9.6, 10.0 (2CH cyclopropyl), 23.4, 23.6 ($2COCH_3$), 83.7 (C_2-oxad.), 147.3 (C=N, oxad.), 166.9, 167.1 (C=O). 8 MS: m/z 251 [M^{+}]; Anal. Cald. for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72%. Found; C, 57.05; H 6.62; N, 16.91%.

5-Acetamido-3-acetyl-2-methyl-2-(2-naphthyl)-2,3-dihydro-1,3,4-oxadiazole (4s): Pale solid (71% isolated yield), m.p. 217°C-220°C. FTIR (KBr, cm^{-1}): 3253 (NH), 3049, 2843 (ArH), 1659, 1648 (2C=O), 1610 (C=N), 1159 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.60 (s, 3H, CH_3 of C_2 oxad.), 2.29-2.33 (s, 6H, $2CH_3$), 7.36-8.45 (m, 7H, arom.), 11.06 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 22.9 ($2CH_3$), 23.6 (CH_3 at C_2-oxad.), 84.3 (C_2-oxad.), 134.9, 132.6, 131.0, 129.5, 128.1, 127.6, 126.9, 126.2, 125.2, 121.5 (10 arom. C), 146.9 (C=N, oxad.), 166.6, 166.9 (2C=O). MS: m/z 311 [M^{+}]; Anal. Cald. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50%. Found; C, 65.29; H 5.69; N, 13.32%.

Antimicrobial evaluation

In vitro antimicrobial effects of 1,3,4-oxadiazoline derivatives were tested against four bacterial strains, namely, *Enterococci*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*., and *Proteus spp*., and the tow fungus, *Aspergillus niger* and *Candida albicans*. The antimicrobial activity was determined using the standard agar diffusion method [21]. Dimethyl sulfoxide acted as a controller, and the test was carried out at a concentration of 100 mg/mL and by adding 50 µL to each disk (i.e., 5 µg/disk) using

DMSO as a solvent. The fungi and bacteria were subcultured in agar and potato dextrose agar media. For antibacterial and antifungal activities, the inhibition zone was compared with the standard drug after incubation at 37ºC and 25ºC for 24 h and 72 h, respectively. The results obtained are presented in **TABLE 1.**

Results and Discussion

Chemical synthesis

The N-carbamoylhydrazones employed, in this study, were prepared via condensation reaction of semicarbazide hydrochloride 1 with the corresponding aldehydes or ketones 2a-s in refluxing methanolic sodium acetate in presence of acetic acid affording semicarbazones 3a-s with yields ranging 85-96%. The treatment of N-carbamoylhydrazones 3a-s with refluxing excess acetic anhydride producing a new series of 3-acetyl-5-acetamido-1,3,4- oxadiazoline derivatives 4a-s (**FIG. 1**) in good yields, after purification by recrystallization using ethanol or methanol. The purity of the compounds was checked by TLC and their elemental analysis, which matched within ± 0.3%-0.4% of the theoretical values.

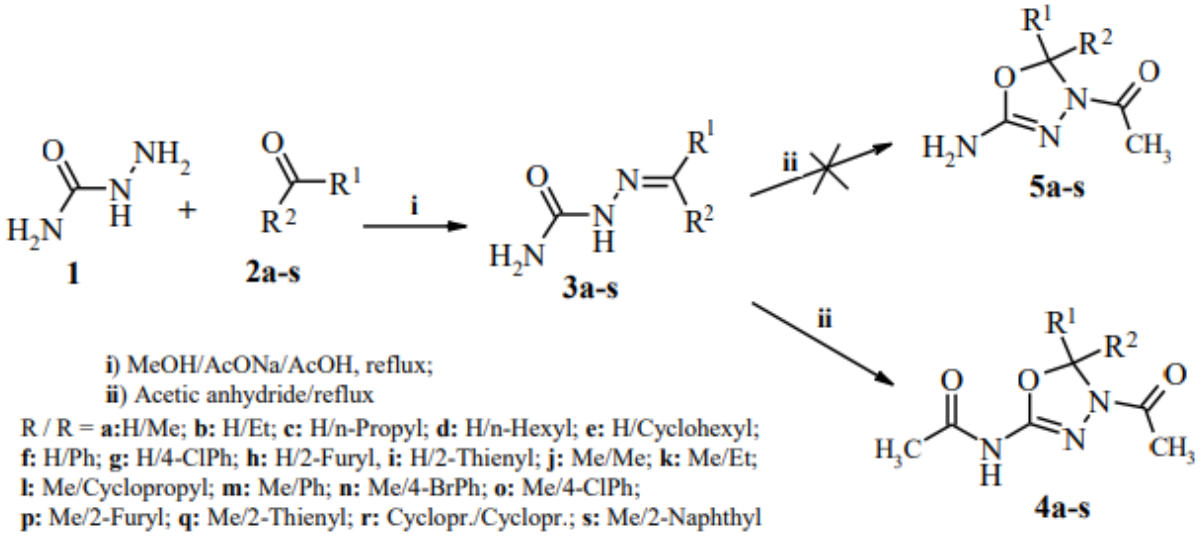

FIG.1. **Synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-s.**

The plausible mechanism of the cyclization of N-carbamoylhydrazones 3a-s to 3-acetyl5-acetamido-2,3-dihydro-1,3,4-oxadiazolines 4a-s. Where induced through the addition of acetic anhydride into imine bond producing intermediate 3', which tautomerized to new intermediate 3" followed with elimination of acetic acid molecule to furnish 1,3,4-oxadiazoline derivatives 4a-s with substitution at amino group of the ring as shown in **FIG. 2** [22]. The formation of non-obtainable 5-amino-1,3,4-oxadiazolines 5a-s dose not observed, further research in this reaction appears promising and necessary.

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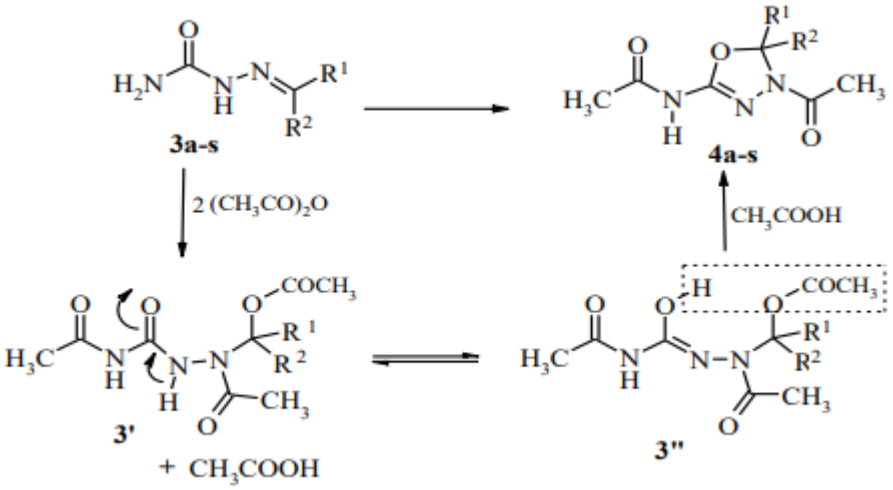

FIG.2. **Suggested mechanism for formation of 1,3,4-oxadiazolines 4a-s**

It is worth mentioning that a series of 2-acylamino-1,3,4-oxadiazoles 5 were directly synthesized by oxidation of 1,4-diacylthiosemicarbazides 6 with aqueous KIO_3 in two hours or by direct acylation of 2-mino-1,3,4-oxadiazoles 7 as shown in **FIG.3.**

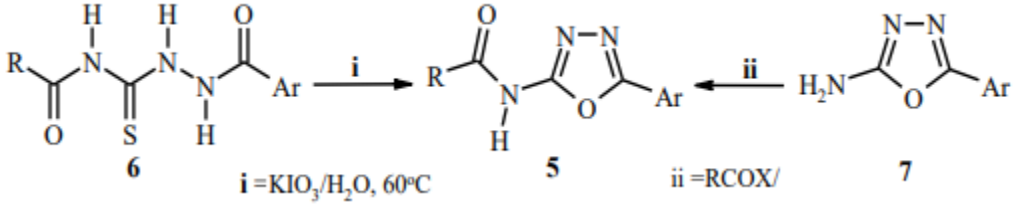

FIG.3. **Synthesis of 2-acylamino-1,3,4-oxadiazole derivatives 5.**

Spectroscopic characterization

The structures of the newly synthesized compounds 4a-s characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra studies. The spectroscopic studies proved the successful acetic anhydride-promoted oxidative cyclization of N-carbamoylhydrazones 3a-s. In their IR spectra of 1,3,4-oxadiazolines 4a-s the characteristic peak for the amine NH_2 at 3400-3300 cm^{-1} and C=O at 1630 cm^{-1}-1650 cm^{-1} of the starting semicarbazones 3a-s completely disappeared from the IR spectra of the obtained products 4a-s. A new C=O stretching of two acetyl groups appeared at 1660-1670 cm^{-1} , amide NH around 3250 cm^{-1} , C-O-C of ring at 1150 cm^{-1}-1170 cm^{-1} , and C=N stretching of dihydro-oxadiazole ring appeared in the range of 1550-1615 cm^{-1} . The second confirmation of the correct structure for compounds 4a-s, comes from their mass spectra were found in good agreement with the newly synthesized compounds.

The ^{1}H-NMR spectra provided clear evidence about the right structure of synthesized compounds 4a-s. The first evidence comes from the disappearance of the characteristic protons of the NH group at 9.0 ppm-10.0 ppm and carbamoyl NH_2 at 5.6-5.8 ppm, in the ^{1}H-NMR spectra of starting compounds 3a-s. The disappearance of the NH protons was accompanied by the appearance of a new signal for amide NH at 10.9-11.5 ppm and tow singlet signals at 2.3-2.5 ppm and these peaks were attributed to $2CH_3$ protons of two acetyl groups indicating the formation of the N-substituted oxadiazoline ring. In compounds, 4a-i the O-CH-N proton was resonated in the range of 6.3-7.1 ppm instead of 8.3-8.9 ppm as shown in precursor hydrazones 3a-i.

The ^{13}C-NMR spectra provided an unambiguous confirmation about the formation of the N-acetyl-2,3-dihydro-1,3,4-oxadiazole ring. In synthesized compounds 4a-s there are a new peaks appeared for acetyl group carbons and this indeed are expected, because they are not part of starting hydrazones 3a-s. where the carbonyl (C=O) of the acetyl groups appeared as two peaks at 166.0, 167.0 ppm due to presence of two acetyl groups, and the other peaks appeared around 23.2/23.7, ppm are assigned to be the peaks of the $2CH_3$ of two acetyl groups. In the same spectra, two major new peaks appeared and both of them confirm the formation of the Nacetyl oxadiazoline ring. The first new and significant peak appeared at 88.0-92.0 ppm which assigned to the carbon-2 (O-C-N) of the 2,3-dihydrooxadiazole ring of compounds 4a-i and the other signal at 83.5-90.5 ppm, is attributed to quaternary carbon of oxadiazoline ring of compounds 4j-s was of special significance in conforming the proposed structure. Which is similar to the reported values of quaternary carbons flanked by heteroatoms in oxadiazole rings [18,23]. The chemical shifts of two ring carbon atoms C-2 and C-5 were dependent on the substituents at the 2- and 5-positions of the 1,3,4-oxadiazoline ring.

Biological activity investigations

The activity of the synthetic compounds against the vulnerable bacteria Enterococci, Escherichia coli, Staphylococcus aureus, Klebsiella spp., and Proteus spp., as well as two species of fungi, Aspergillus niger and Candida albicans, was assessed using the standard nutrient agar disc diffusion method [24,25]. One gram of each compound was dissolved in 10 ml of DMSO. Thus, 100 mg/ml of stock was obtained as a standard concentration of compounds. The compounds were examined at a concentration of 1 mg mL^{-1} in a solution of Dimethyl Sulfoxide (DMSO), and all tests were implemented in triplicates and the average diameter of the inhibitory zone was measured in millimeters.

In comparison to well-known antibacterial and antifungal chemicals like tetracycline and fluconazole, the results showed that all of the tested compounds shown a significant amount of action against bacteria and fungi. NCCLS classifies inhibition zones for tetracycline and fluconazole as “highly active” (inhibition zone ≥ 19 mm), “moderately active” (inhibition zone 15 mm–18 mm), or “inactive” (inhibition zone ≤ 14 mm) [25]. The activity against both bacteria and fungus was moderately enhanced by the addition of the N-acetyl moiety **(TABLE 1)**. Future medicinal chemists may use the results of the current work to develop and create molecules with a similar structure with greater biological efficacy.

Tab. 1. **Antimicrobial screening results of the tested compounds 4a-s.**

Diameter of the Inhibition Zone in mm*							
Cpd. No.	**Antibacterial Activity**			**Antifungal Activity**			
	Enterococci	***Escherichia coli***	***Staphyloaureus***	***Klebsiella spp***	***Proteus spp***	***Candida albicans***	***Aspergillus niger***
4a	18	16	18	16	18	16	19
4b	19	18	19	17	17	19	16
4c	18	16	17	15	16	18	16
4d	17	18	19	17	16	15	14
4e	16	19	16	19	15	19	13
4f	17	16	18	17	16	17	19
4g	19	18	17	19	16	18	16
4h	18	16	19	18	19	16	18
4i	16	19	16	19	16	19	17
4j	18	16	18	16	17	16	19
4k	16	17	17	15	16	16	18
4l	17	18	17	16	15	17	18
4m	19	15	17	18	16	18	16
4n	16	16	17	18	19	16	17
4o	17	19	16	19	16	17	19
4p	18	16	17	19	16	18	19
4q	18	15	15	17	16	18	16
4r	17	16	17	18	19	16	18
4s	17	19	16	19	18	19	18
Tet.[a] Flu.[b]	25	21	23	22	24	26	25
DMSO	--	--	--	--	--	--	--

Note: * Calculated as average of three values. [a]Tetracycline, [b]Fluconazole

Conclusion

New series of novel functionalized 1,3,4-oxadiazolines 4a-s were synthesized upon the treatment of carbamoylhydrazones of aldehydes 3a-i, ketones 3j-s with acetic anhydride under refluxing conditions and evaluated for their in vitro antibacterial, and antifungal activities. From the screening results, it found to possess various antimicrobial activities towards all the microorganisms tested. The results confirm that, the antimicrobial activity is strongly dependent on the nature of the substituents on oxadiazole ring and the presence of acetyl group at position-3 and acetamido group at position-2 of the ring enhance their biological activities. To better understand the chemical mechanism causing the activity seen, further research is needed to fully understand the remarkable

features of this novel family of antimicrobial compounds. A more thorough investigation is also necessary to identify new physicochemical and biological factors in order to better understand the relationship between structure and activity and to maximize the efficiency of this group of molecules.

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