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Synthesis, Characterization and Antibiotics of Novel 3-Butanoyl-2,3-dihydro-1,3,4-oxadiazole Derivatives

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Abstract
A new series of 3-butanoyl-1,3,4-oxadiazoline derivatives were synthesized via oxidative cyclization reaction of different N-carbamoylhydrazoneswith butyric anhydride.The structures of obtained compounds were confirmed by IR, MS, ^{1}H NMR, ^{13}C NMR and Elemental analysis methods andare 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 (Euterococci, Escherichia coli, Staphylococcus aureus, Klebsiellaspp, 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 *3-Butanoyl-1,3,4-oxadiazolines; N-carbamoylhydrazones;Oxidative cyclization;Antimicrobial activity.*

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I. NTRODUCTION

Research and development of potent and effective antimicrobials is one of the most important factors in advances in treatment, not only in the control of serious infections, but also in the prevention and treatment of some other infectious complications. In recent years, organic chemists have been interested in designing and manufacturing new and therapeutically effective compounds, useful for alleviating various disorders and diseases. Among the important drugs responsible for antimicrobial activity, azoles are still the most effective against pathogenic microorganisms. Oxadiazoles are a class of heterocyclic organic compounds known for their remarkable therapeutic pharmaceutical potentials. Thousands of oxadiazoles and its derivatives have been synthesized and evaluated for various antimicrobial and enzyme inhibition activities. Oxadiazoles possess interesting anti-inflammatory [1,2], fungicidal [3], insecticidal [4], herbicidal [5], antibacterial [6], antitumor [7], antitubercular [8], antiviral, anticonvulsant and analgesic activities [9]. Many compounds containing 1,2,4-, 1,3,4-oxadiazole moiety are useful used drugs (Figure) such as Zibotentan, Tiodazosin, Raltegravir, Nesapidil, Furamizole, Tiodazosin,Fasipolen, Ataloren, and Oxolamine. The 1,2,4-oxadiazole derivatives are part of natural products and many biologically important molecules [10]. Many reviews recover the synthetic methodologies of oxadiazoles, their medicinal and energetic applications [11-14].1,3,4-Oxadiazole derivatives were generally synthesized from acylhydrazones using different oxidizing agents as lead tetraacetate (LTA) in CH_2Cl_2 [15], phenyliodine (III) diacetate (PIDA) in ethanol [16], electrolytic oxidation in methanolic sodium acetate ($AcONa/CH_3OH$) involving intramolecular cyclization to give 2-methoxy-1,3,4-oxadiazoline [17]. Recently Ultrasound-assisted synthesis of 2-amino-1,3,4-oxadiazoles through NBS-mediated oxidative cyclization of semicarbazones has been reported [18].

Using acid anhydride was the most common procedure of cyclization of acylhydrazones. The acetic anhydride (AA) is frequently used as dehydrant in these kinds of reactions in many literatures. The treated of acylhydrazones with (AA) found to give 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines with good yield [19-21]. 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 reported here the synthesis of some novel 3-butanoyl-1,3,4-oxadiazolines and their antimicrobial activity were investigated.

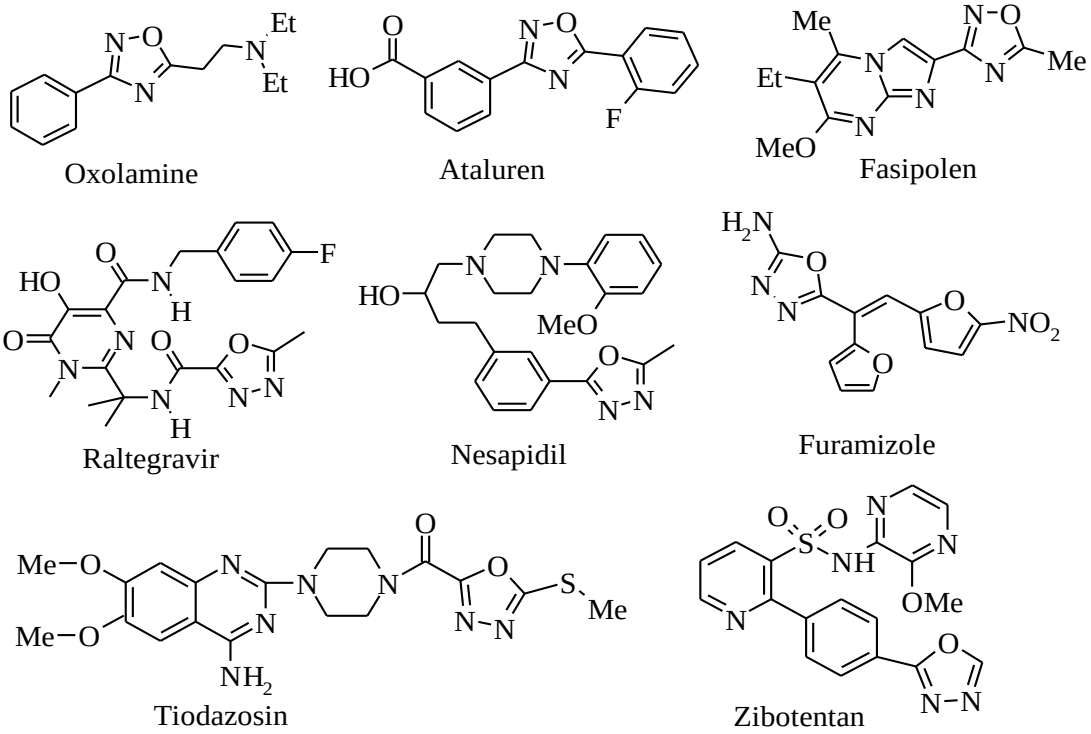


Figure: Examples of drugs molecules containing oxadiazole heterocycle

II. MATERIALS AND METHODS

Chemicals and apparatus

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 and ^{13}C NMR spectra were recorded in (DMSO-d_6) on JEOL 500 NMR spectrometer (GmbH, Freising, Germany). 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 (EI) mass spectra were measured 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. 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 Merck Co.

Chemical methods

General Procedure for Synthesis ofCarbamoylhydrazones 3a-s.

A mixture of semicarbazide hydrochloride **1** (0.015 mol), aldehydes or ketones **2a-o** (0.015 mol), sodium acetate (0.015 mol) and few drops of glacial acetic acid in methanol (30 mL) was stirred under reflux until reaction had completed (1-2 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.[18,22,23]

General Procedure for Synthesis of 1,3,4-Oxadiazolines 4a-s.

A mixture of carbamoyl hydrazones **3a-s** (0.0015-0.002 mol) and excessive butyric anhydride (15-25 mL) was stirred under reflux in oil bath at 120-140 ^{o}C for 3-4 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 followed by water and dried.In some cases the reaction mixture left for 2-4 days to vaporize the excess anhydride or removed invacuo, and the residue washed with potassium bicarbonate solution and finally with water and dried. In all casesthe resulting crude solid product was recrystallized fromethanolor ethyl acetate and air-dried to afford 1,3,4-oxadiazolines**4a-s**.

3-Butanoyl-5-butanoylamino-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4a).
White solid (68% isolated yield), m.p. 152-154 °C. FTIR (KBr, cm^{-1}): 3254 (NH), 1670, 1667 (2C=O), 1610 (C=N), 1159 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.98-1.01 (t, 6H, $2CH_3$), 1.54 (d, 3H, $2CH_3$of C_2-oxad.),1.60-1.64 (m,4H, $2CH_2$), 2.32-2.35 (t, 4H, $2CH_2$), 4.28 (q, 1H, CHof C_2-oxad.), 11.82 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ =13.0, 13.1 ($2CH_3$ of butyryl),18.6, 18.8 ($2CH_2$ of butyryl), 24.6 (CH_3), 48.8, 48.9 ($2CH_2$ of butyryl), 91.8 (C_2-oxad.), 151.3 (C=N, oxadiazole ring), 167.5, 166.9 (2C=O).MS: *m/z* 226 [$M^{+\cdot}$];Anal. Cald. for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38%. Found: C, 58.11; H 7.84; N, 12.20%.
3-Butanoyl-5-butanoylamino-2-phenyl-2,3-dihydro-1,3,4-oxadiazole (4b).
White solid (65% isolated yield), m.p. 169-172 °C. FTIR (KBr, cm^{-1}): 3256 (NH), 1671, 1663 (2C=O), 1608 (C=N), 1161 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.96-1.00 (t, 6H, $2CH_3$), 1.60-1.63 (m, 4H, $2CH_2$), 2.31-2.35 (t, 4H, $2CH_2$), 7.32-7.93 (m, 5H, arom.), 8.12 (s, 1H, CH at C_2-oxad.), 11.53 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0/13.3 ($2CH_3$ of butyryl), 18.5/18.9 ($2CH_2$ of butyryl), 48.5/48.8 ($2CH_2$ of butyryl), 92.3 (C_2-oxad.),134.2, 130.8, 128.7, 126.7 (4arom. C), 149.6 (C=N, oxad.), 166.9/167.4 (2C=O).MS: *m/z* 288 [M^{+}];Anal. Cald. for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72%. Found; C, 66.89; H 7.21; N, 9.43%.
3-Butanoyl-5-butanoylamino-2-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole (4c).
White solid (67% isolated yield), m.p. 176-178 °C. FTIR (KBr, cm^{-1}): 3258 (NH), 1668, 1664 (2C=O), 1605 (C=N), 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.99-1.04 (t, 6H, $2CH_3$), 1.58-1.62 (m, 4H, $2CH_2$), 2.31-2.36 (t, 4H, $2CH_2$), 5.54 (s, 1H, OH), 7.21-7.96 (m, 4H, arom.), 8.09 (s, 1H, CH at C_2-oxad.), 11.52 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 ($2CH_3$ of butyryl), 18.9 (CH_2 of butyryl), 48.8 ($C=OCH_2$ of butyryl), 92.7 (C_2-oxad.), 139.7, 131.8, 129.2, 128.1, 125.2 (5arom. C), 149.6 (C=N, oxad.), 157.7 (C-OH), 166.9/166.4 (2C=O).MS: *m/z* 304 [$M^{+\cdot}$];Anal. Cald. for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20%. Found; C, 62.84; H 6.83; N, 9.00%.
3-Butanoyl-5-butanoylamino-2-(2-furyl)-2,3-dihydro-1,3,4-oxadiazole (4d).
White solid (53% isolated yield), m.p. 187-189 °C. FTIR (KBr, cm^{-1}): 3260 (NH), 1660, 1657 (2C=O), 1610 (C=N), 1154 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.99-1.03 (t, 6H, $2CH_3$), 1.57-1.63 (m, 4H, $2CH_2$), 2.15-2.33 (t, 4H, $2CH_2$), 7.56-8.20 (m, 4H, arom.), 8.46 (s, 1H, CH at C_2-oxad.), 11.46 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0/13.4 ($2CH_3$ of butyryl), 18.9/19.1 ($2CH_2$ of butyryl), 48.3-48.8 ($2CH_2$ of butyryl), 92.4(C_2-oxad.), 145.7, 139.4, 114.2, 113.1 (4 C furan ring), 149.7 (C=N, oxad.), 166.7/166.1 (2C=O).MS: *m/z* 264 [M^{+}];Anal. Cald. for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.02%. Found; C, 68.81; H 6.34; N, 9.81%.
3-Butanoyl-5-butanoylamino-2-(2-thienyl)-2,3-dihydro-1,3,4-oxadiazole (4e).
White solid (65% isolated yield), m.p. 193-195 °C. FTIR (KBr, cm^{-1}): 3254 (NH), 1665, 1659 (2C=O), 1611 (C=N), 1152 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.97-1.02 (t, 6H, $2CH_3$), 1.59-1.63 (m, 4H, $2CH_2$), 2.21-2.33 (t, 4H, $2CH_2$), 7.42-8.17 (m, 4H, arom.), 8.45 (s, 1H, CH at C_2-oxad.), 11.62 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 48.8 ($C=OCH_2$ of butyryl), 92.6 (C_2-oxad.), 144.8, 140.4, 119.6, 114.2 (4 C thiophene ring), 146.9 (C=N, oxad.), 166.9 (C=O).MS: *m/z*280 [$M^{+\cdot}$];Anal. Cald. for $C_{13}H_{16}N_2O_3S$: C, 55.70; H, 5.75; N, 9.99%. Found; C, 55.43; H 5.53; N, 9.75%.
3-Butanoyl-5-butanoylamino-2-ethyl-2-methyl-2,3-dihydro-1,3,4-oxadiazole(4f).
Pale yellow solid (68% isolated yield), m.p. 179-181 °C. FTIR (KBr, cm^{-1}): 3254 (NH), 1669, 1663 (2C=O), 1598 (C=N), 1162 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.94-1.00 (t, 6H, $3CH_3$), 1.24 (s, 3H, CH_3 of ethyl), 1.54 (s, 3H, CH_3at C_2-oxad.), 1.60-1.63 (m, 4H, $2CH_2$), 2.12-2.35 (t, 4H, $2CH_2$), 2.63 (d, 2H, CH_2 of ethyl), 12.24 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0/13.3 ($2CH_3$ of butyryl), 13.6 (CH_3 of ethyl), 18.3/18.9 ($2CH_2$ of butyryl), 21.4 (CH_2 at C_2-oxad.), 24.7 (CH_3 at C_2-oxad.), 48.3/48.8 ($2CH_2$ of butyryl), 99.3(quaternary C_2-oxad.), 154.1 (C=N, oxad.), 166.9/167.3 (2C=O).MS: *m/z* 240 [$M^{+\cdot}$];Anal. Cald. for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 10.66%. Found; C, 60.25; H 8.61; N, 10.87%.
2-Cyclopropyl-3-Butanoyl-5-butanoylamino-2-methyl-2,3-dihydro-1,3,4oxadiazole (4g).
Yellow solid (60% isolated yield), m.p. 166-169 °C. FTIR (KBr, cm^{-1}): 3258 (NH), 1672, 1668 (2C=O), 1604 (C=N), 1162 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.54-0.69 (m, 4H, $2CH_2$ of cyclopropyl), 0.98-1.03 (t, 6H, $2CH_3$), 1.28-1.34 (m, 1H, CH of cyclopropyl), 1.54 (s, 3H, CH_3 of C_2oxad.), 1.61/1.65 (m, 4H, $2CH_2$), 2.31-2.36 (t, 4H, $2CH_2$), 7.16-7.94 (m, 4H, arom.), 12.24 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0/13.4 ($2CH_3$ of butyryl), 13.3 (CH_3 of ethyl), 18.6/18.9 ($2CH_2$ of butyryl), 22.1 (CH_2 at C_2-oxad.), 24.6 (CH_3 at C_2-oxad.), 48.3/48.8 ($2CH_2$ of butyryl), 99.7 (quaternary C_2-oxad.), 153.4 (C=N, oxad.), 166.8, 167.2(C=O).MS: *m/z* 252 [$M^{+\cdot}$]; Anal. Cald. for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10%. Found; C, 62.15; H 7.76; N, 10.87%.
2-(4-Bromophenyl)-3-Butanoyl-5-butanoylamino-2-methyl-2,3-dihydro-1,3,4-oxa-diazole (4h).
Pale yellow solid (62% isolated yield), m.p. 167-169 °C. FTIR (KBr, cm^{-1}): 3257 (NH), 1666, 1661 (2C=O), 1593 (C=N), 1157 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.98-1.05 (t, 6H, $2CH_3$), 1.56 (s, 3H, CH_3 of C_2oxad.), 1.59-1.63 (m, 2H, $2CH_2$), 2.32-2.38 (t, 4H, $2CH_2$), 7.26-7.90 (m, 4H, arom.), 12.34 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0, 13.3 ($2CH_3$ of butyryl), 18.2, 18.9 ($2CH_2$ of butyryl), 24.6 (CH_3 at C_2-

oxad.), 48.5, 48.8 ($2CH_2$ of butyryl), 99.6 (quaternary C_2-oxad.), 133.7, 131.5, 128.4, 127.2 (4arom. C), 153.9 (C=N, oxad.), 166.4, 166.9 (2C=O).MS: *m/z*367 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{19}BrN_2O_3$: C, 52.33; H, 5.21; N, 7.63%. Found; C, 52.61; H 5.42; N, 7.42%.

3-Butanoyl-5-butanoylamino-2-(2-furyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4i).

White solid (55% isolated yield), m.p. 206-208 °C. FTIR (KBr, cm^{-1}): 3254 (NH), 1664, 1658 (2C=O), 1602 (C=N), 1159 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01-1.06 (t, 6H, $2CH_3$), 1.54(s, 3H, CH_3 of C_2oxad.), 1.59-1.63 (m, 4H, $2CH_2$), 2.33-2.38 (t, 4H, $2CH_2$), 7.64-8.27 (m, 3H, arom.), 12.60 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.1, 13.5 ($2CH_3$ of butyryl), 18.4, 18.9 ($2CH_2$ of butyryl), 24.2 (CH_3 at C_2-oxad.), 48.5, 48.8 ($2CH_2$ of butyryl), 100.2 (quaternary C_2-oxad.), 139.2, 129.6, 128.4, 125.8 (4arom. C), 147.9 (C=N, oxad.), 166.3, 167.0 (2C=O).MS: *m/z*278 [$M^{+\cdot}$];Anal. Cald. for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07%. Found; C, 60.16; H 6.31; N, 9.86%.

3-Butanoyl-5-butanoylamino-2-(2-thienyl)-2-methyl-2,3-dihydro-1,3,4-oxadiazole (4j).

Pale brown solid (56% isolated yield), m.p. 198-201 °C. FTIR (KBr, cm^{-1}): 3255 (NH), 1665, 1656 (2C=O), 1605 (C=N), 1165 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.00-1.04 (t, 6H, $2CH_3$), 1.53 (s, 3H, CH_3 of C_2oxad.), 1.60-1.63 (m, 4H, $2CH_2$), 2.29-2.36 (t, 4H, $2CH_2$), 7.56-7.97 (m, 3H, arom.) 12.42 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.8, 13.0 ($2CH_3$ of butyryl), 18.3, 18.9 ($2CH_2$ of butyryl), 24.3 (CH_3 at C_2-oxad.), 48.5, 48.8 ($2CH_2$ of butyryl), 100.7 (quaternary C_2-oxad.), 130.8, 129.9, 128.1, 125.2 (4 C thiophene ring), 147.6 (C=N, oxad.), 166.4, 166.9 (2C=O).MS: *m/z* 294 [$M^{+\cdot}$];Anal. Cald. for $C_{14}H_{18}N_2O_3S$: C, 57.12; H, 6.16; N, 9.52%. Found; C, 56.83; H 6.37; N, 9.31%.

3-Butanoyl-5-butanoylamino-1,3,4-oxadiazaspiro[4.4]non-2-ene (4k).

White solid (62% isolated yield), m.p. 178-180 °C. FTIR (KBr, cm^{-1}): 3250 (NH), 1665, 1658 (2C=O), 1601 (C=N), 1156 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.98-1.02 (t, 6H, $2CH_3$), 1.59-1.63 (m, 4H, $2CH_2$), 1.67-1.88 (m, 8H, $4CH_2$ cyclopentane), 2.28-2.33 (t, 4H, $2CH_2$), 12.24 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.7, 13.1 ($2CH_3$ of butyryl), 18.3, 18.9 ($2CH_2$ of butyryl), 38.4, 36.1, 24.4, 23.2 ($4CH_2$ cyclopentane),48.5, 48.9 ($2CH_2$ of butyryl), 100.8 (spiro C_2-oxad.), 156.5 (C=N, oxad.), 166.5, 167.6 (2C=O).MS: *m/z*252 [$M^{+\cdot}$];Anal. Cald. for $C_{13}H_{20}N_2O_3$:C, 61.88; H, 7.99; N, 11.10%. Found; C, 62.15; H 8.16; N, 10.92%.

3-Butanoyl-5-butanoylamino-1,3,4-oxadiazaspiro[4.5]dec-2-ene (4l).

Off-white solid (66% isolated yield), m.p. 217-219 °C. FTIR (KBr, cm^{-1}): 3224 (NH), 1663, 1665 (2C=O), 1567 (C=N),1249 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.97-1.02 (t, 6H, $2CH_3$), 1.54-2.14 (m, 10H, 5CH2 cyclohexane), 1.58-1.63 (m, 4H, $2CH_2$), 2.27-2.33 (t, 4H, $2CH_2$), 12.42 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.6, 13.0 ($2CH_3$ of butyryl), 18.3, 18.9 ($2CH_2$ of butyryl), 33.1, 31.2, 24.6, 23.2, 21.4 ($5CH_2$ cyclohexane), 48.3, 48.8 ($2CH_2$ of butyryl), 100.4 (spiro C_2-oxad.), 154.2 (C=N, oxad.), 166.8, 167.3 (2C=O).MS: *m/z*266 [$M^{+\cdot}$];Anal. Cald. for $C_{14}H_{22}N_2O_3$:C, 63.14; H, 8.33; N, 10.52%. Found; C, 62.89; H 8.11; N, 10.73%.

3-Butanoyl-5-butanoylamino-8-methyl-1,3,4-oxadiazaspiro[4.5]dec-2-ene (4m).

White solid (62% isolated yield), m.p. 196-198 °C. FTIR (KBr, cm^{-1}): 3248 (NH), 1665, 1657 (2C=O), 1611 (C=N), 1159 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.96 (d, 3H, C-CH_3), 1.01-1.06 (t, 6H, $2CH_3$), 1.59-1.63 (m, 4H, $2CH_2$), 1.16-2.11 (m, 9H, $4CH_2$, CH cyclohexane), 2.30-2.33 (t, 4H, $2CH_2$), 12.61 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.6, 13.0 ($2CH_3$ of butyryl), 18.5, 18.9 ($2CH_2$ of butyryl), 48.3, 48.8 ($2CH_2$ of butyryl), 31.4 (CH_3 at cyclohexane), 33.3, 28.8, 24.7, 24.3 ($4CH_2$ cyclohexane), 38.4 (CH of cyclohexane), 100.9 (spiro C_2-oxad.),154.3 (C=N, oxad.), 166.7, 167.3 (C=O). MS: *m/z*280 [$M^{+\cdot}$]; Anal. Cald. for $C_{15}H_{24}N_2O_3$: C, 64.26; H, 8.63; N, 9.99%. Found; C, 64.55; H 8.81; N, 10.18%.

3-Butanoyl-5-butanoylamino-8-t-butyl-1,3,4-oxadiazaspiro[4.5]dec-2-ene (4n).

White solid (70% isolated yield), m.p. 180-182 °C. FTIR (KBr, cm^{-1}): 3247 (NH), 1660, 1656 (2C=O), 1597 (C=N), 1149 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.87 (s, 9H, $3CH_3$ t-Bu), 0.98-1.02 (t, 6H, $2CH_3$), 1.58-1.63 (m, 4H, $2CH_2$), 1.10-2.13 (m, 9H, $4CH_2$, CH cyclohexane), 2.28-2.33 (t, 2H, $2CH_2$), 12.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.9, 13.2 ($2CH_3$ of butyryl), 18.5, 18.9 ($2CH_2$ of butyryl), 48.3, 48.8 ($2CH_2$ of butyryl), 38.1,(CH_3 at cyclohexane), 33.2, 28.6, 24.6, 24.3 ($4CH_2$ cyclohexane), 38.5 (CH of cyclohexane), 100.7 (spiro C_2-oxad.), 154.3 (C=N, oxad.), 166.8, 167.3 (2C=O).MS: *m/z* 322 [$M^{+\cdot}$]; Anal. Cald. for $C_{18}H_{30}N_2O_3$: C, 67.05; H, 9.38; N, 8.69%. Found; C, 66.84; H 9.57; N, 8.51%.

3-Butanoyl-5-butanoylamino-8-methyl-1,3,4,8-oxatriazaspiro[4.5]dec-2-ene (4o).

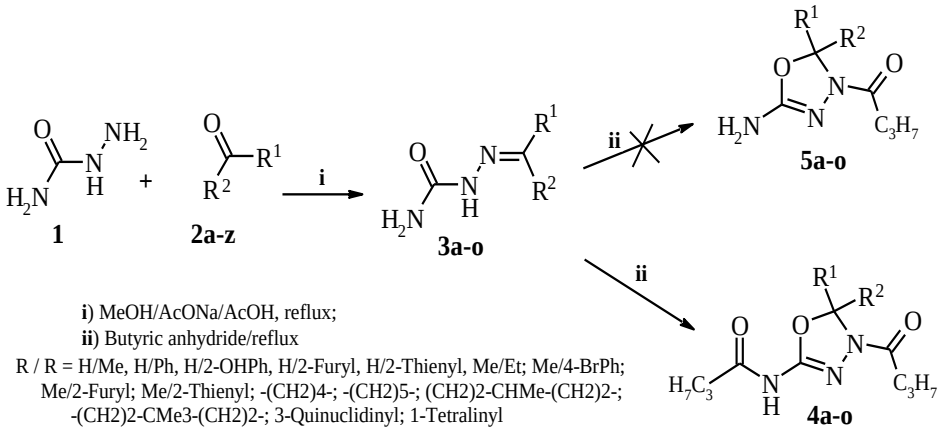
Pale yellow solid (58% isolated yield), m.p. 207-210 °C. FTIR (KBr, cm^{-1}): 3251 (NH), 1672, 1664 (2C=O), 1611 (C=N), 1160 (C-O-C).^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.97-1.02 (t, 6H, $2CH_3$), 1.57-1.63 (m, 4H, $2CH_2$), 1.81-2.84 (m, 8H, $4CH_2$ cyclohexane), 2.30-2.36 (t, 4H, $2CH_2$), 2.46 (s, 3H, NCH_3), 12.45 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0, 13.2 ($2CH_3$ of butyryl), 18.6, 18.9 ($2CH_2$ of butyryl), 31.6 ($2CH_2$), 46.3 (NCH_3), 48.4-48.8 ($2CH_2$ of butyryl), 53.5 ($2CH_2$), 100.8 (spiro C_2-oxad.), 154.5 (C=N, oxad.), 166.7, 167.2 (2C=O). MS: *m/z*281 [M^+]; Anal. Cald. for $C_{14}H_{23}N_3O_3$: C, 59.77; H, 8.24; N, 14.93%. Found; C, 67.87; H 7.61; N, 14.01%.

3-Butanoyl-5-butanoylamino-8-isopropyl-1,3,4,8-oxatriazaspiro[4.5]dec-2-ene (4p).
Off White solid (63% isolated yield), m.p. 175-177 °C. FTIR (KBr, cm^{-1}): 3252 (NH), 1670, 1663 (2C=O), 1613 (C=N), 1158 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.97-1.01 (t, 6H, $2CH_3$), 1.26 (d, 6H, $2CH_3$isopr.), 1.58-1.63 (m, 4H, $2CH_2$), 1.86-3.15 (m, 8H, $4CH_2$ cyclohexane), 2.30-2.36 (t, 4H, $2CH_2$), 2.43 (m, 1H, NCH),12.36 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0, 13.2 ($2CH_3$ of butyryl), 18.5, 18.9 (CH_2 of butyryl), 27.8 ($2CH_3$isopr.), 31.2 ($2CH_2$), 47.3 (NCH), 48.4, 48.8 ($2CH_2$ of butyryl), 53.3 ($2CH_2$), 101.4 (spiro C_2-oxad.), 153.6 (C=N, oxad.), 167.0, 167.3 (2C=O). MS: *m/z* 309 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{27}N_3O_3$: C, 62.11; H, 8.80; N, 13.58%. Found; C, 61.85; H 8.62; N, 13.40%.
8-Benzyl-3-butanoyl-5-butanoylamino-1,3,4,8-oxatriazaspiro[4.5]dec-2-ene (4q).
White solid (64% isolated yield), m.p. 164-166 °C. FTIR (KBr, cm^{-1}): 3247 (NH), 1672, 1665 (2C=O), 1609 (C=N), 1162 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.99-1.04 (t, 6H, $3CH_3$), 1.58-1.63 (m, 4H, $2CH_2$), 1.74-2.86 (m, 8H, $4CH_2$ cyclohexane), 2.29-2.33 (t, 4H, $2CH_2$), 3.36 (s, 2H, NCH_2), 7.20-8.13 (m, 5H, arom.), 12.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 12.8, 13.1 ($2CH_3$ of butyryl), 18.7, 18.9 (CH_2 of butyryl), 32.2 ($2CH_2$), 48.6, 48.8 ($2CH_2$ of butyryl), 50.2 ($PhCH_2$), 53.6 ($2CH_2$), 101.6 (spiro C_2-oxad.), 131.8, 128.4, 127.2, 125.8 (4arom. C), 153.9 (C=N, oxad.), 167.1, 167.3 (2C=O). MS: *m/z* 357 [$M^{+\cdot}$]; Anal. Cald. for $C_{20}H_{27}N_3O_3$: C, 67.20; H, 7.61; N, 11.76%. Found; C, 67.43; H 7.40; N, 11.97%.
3'-Butanoyl-5'-butanoylamino-3H'-spiro[4-azabicyclo[2.2.2]octane-2,2'-[1,3,4]oxa-diazole](4r).
White solid (46% isolated yield), m.p. 243 °C dec.FTIR (KBr, cm^{-1}): 3245 (NH), 1670, 1664 (2C=O), 1612 (C=N), 1161 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.00-1.03 (t, 6H, $2CH_3$ butyryl), 1.6-1.65 (m, 4H, $2CH_2$ butyryl), 1.70-2.02 (m, 4H, $2CH_2$), 2.29-2.33 (t, 4H, $2CH_2$ butyryl), 2.40-2.64 (m, 1H, CH), 3.28-3.51 (m, 6H, $3CH_2$),12.56 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0, 13.2 ($2CH_3$ of butyryl), 18.6, 18.9 (CH_2 of butyryl), 27.6, 28.1 ($2CH_2$), 32.6 (CH), 45.9, 46.4 ($2CH_2$),48.6, 48.8 ($2CH_2$ of butyryl), 56.3 (CH_2), 103.1 (spiro C), 147.3 (C=N, oxad.),166.9, 167.1 (C=O).MS: *m/z*293 [M^+];Anal. Cald. for $C_{15}H_{23}N_3O_3$: C, 61.41; H, 7.90; N, 14.32%. Found; C, 61.05; H 8.15; N, 14.09%.
3'-Butanoyl-5'-butanoylamino-3,4-dihydro-2H,3H'-spiro[naphthalene-1,2,-[1,3,4]-oxadiazole] (4s).
Pale solid (51% isolated yield), m.p. 217-220 °C. FTIR (KBr, cm^{-1}): 3254 (NH), 1673, 1668 (2C=O), 1610 (C=N), 1159 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02-1.07 (t, 6H, $2CH_3$), 1.13-2.15 (m, 6H, $3CH_2$ tetralinyl),1.61-1.66 (m, 4H, $2CH_2$), 2.29-2.35 (t, 4H, $2CH_2$), 7.26-7.81 (m, 4H, arom.), 11.86 (s, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0, 13.2 ($2CH_3$ of butyryl), 18.7, 18.9 ($2CH_2$ of butyryl), 25.7 ($2CH_2$), 33.8 (CH), 48.5, 48.7 ($2CH_2$ of butyryl), 50.4 ($2CH_2$), 55.7 (CH_2), 102.3 (spiro C), 133.2, 131.0, 128.4, 127.2 (4arom. C), 146.9 (C=N, oxad.), 166.6, 166.9 (C=O).MS: *m/z* 314 [$M^{+\cdot}$];Anal. Cald. for $C_{18}H_{22}N_2O_3$: C, 68.77; H, 7.05; N, 8.91%. Found; C, 68.42; H 6.82; N, 9.15%.

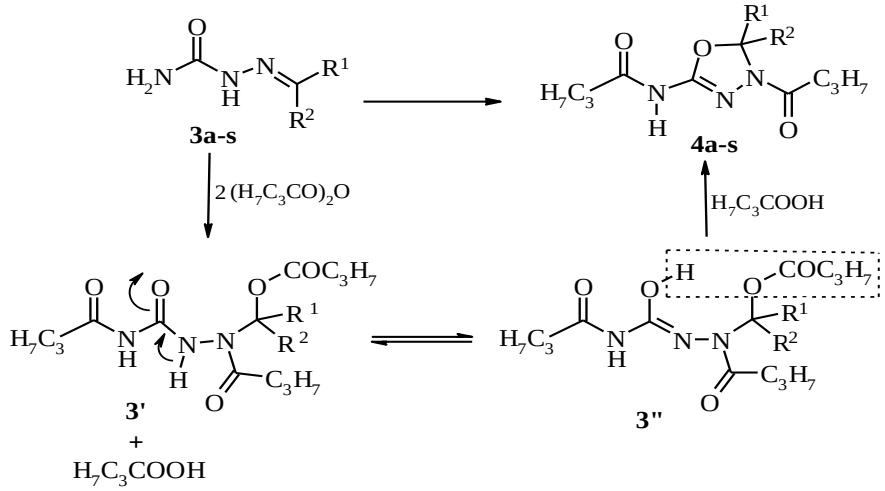
III. RESULT AND DISCUSSION

Chemical synthesis

The precursor's aldehydes semicarbazones or ketones semicarbazones employed, in this study, were prepared via reaction of semicarbazide hydrochloride **1** with the corresponding carbonyl compounds **2a-s** (aldehydes or ketones) in refluxing methanolic sodium acetate in presence of acetic acid producing semicarbazones **3a-s** (Scheme 1) with yields ranging 85-96%. The treatment of N-carbamoylhydrazones **3a-s** with refluxing excess butyric anhydride furnished a new series of 3-butanoyl-5-butanoylamino-1,3,4-oxadiazoline derivatives **4a-s** (Scheme1) 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. The plausible mechanism of the cyclization of carbamoyl hydrazones **3a-s** to 3-butanoyl-2,3-dihydro-1,3,4-oxadiazolines **4a-s**. Where induced through the addition of butyric anhydride into imine bond producing intermediate **3',** which tautomerized to new intermediate **3"** followed with elimination of butyric acid molecule to furnish 1,3,4-oxadiazoline derivatives **4a-s** with substitution on amino group of the ring as shown in Scheme 2 [24]. 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.

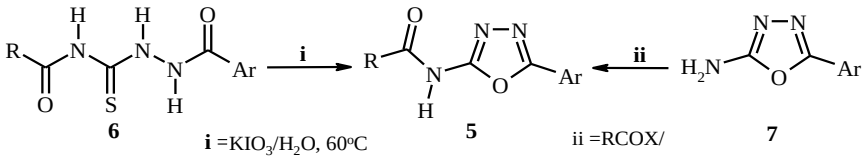


Scheme 1: Schematic synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-s.



Scheme 2: Plausible 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 acylation of 2-mino-1,3,4-oxadiazoles **7** as shown in Scheme 3. The structures of the newly synthesized compounds **4a-s** characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra studies. The synthesized compounds were found in good agreement with their spectral data.



Scheme 3: Synthesis of 2-acylamino-1,3,4-oxadiazole derivatives 5.

Spectroscopic Characterization

The spectroscopic studies proved the successful butyric 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 1630-1650 cm^{-1} of the starting carbamoylhydrazones **3a-s** completely disappeared from the IR spectra of the obtained products **4a-s**. A new C=O stretching of two N-butanoyl groups appeared at 1660-1670 cm^{-1}, amide NH arround 3250 cm^{-1},C-O-C of ring at 1150-1170 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 9.0-10.0 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 11.5-12.5 ppm and three signals at 2.3-2.5 (t), 1.6-1.7(m) and 1.0-0.95(t) ppm and these peaks were assigned to the protons ($CH_2CH_2CH_3$) of two butyryl groups indicating the formation of the N-substituted oxadiazoline ring. In compounds, **4a-e** the O-CH-N proton was resonated with more down field around 7.0 ppm instead of 8.5 ppm as shown in precursor hydrazones **3a-e**. For compounds **4k-s** the peaks for the cycloalkane ring were resonated in the aliphatic region of the spectra on the range of 2.8-1.1 ppm, in addition to aromatic protons. The ^{13}C-NMR spectra provided an unambiguous confirmation about the formation of the N-butyryl-2,3-dihydro-1,3,4-oxadiazole ring. In synthesized compounds **4a-s** there are a new peaks appeared for butanoyl group carbons and this indeed are expected, because they are not part of starting hydrazones **3a-s**. where the carbonyl (C=O) of the butanoyl groups appeared as two peaks at 166.0, 167.0 ppm due to presence of two butyryl groups, and the other peaks appeared as doublet around 48.5/48.8, 18.5/19.0 and 13.0/13.4 ppm are assigned to be the peaks of the $CH_2CH_2CH_3$ of two butanoyl groups. In the same spectra, two major new peaks appeared and both of them confirm the formation of the N-butanoyl oxadiazoline ring. The first new and significant peak appeared at 91.0-92.0 ppm which assigned to the carbon-2 (O-C-N) of the 2,3-dihydro-oxadiazole ring of compounds **4a-e** and the other signal at 100.0-105.0 ppm, is attributed to spiro-carbon of oxadiazoline ring of compounds **4f-s** was of special significance in conforming the proposed structure. Which is similar to the reported values of spiro-carbons flanked by heteroatoms in oxadiazole rings [25]. 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.

Antimicrobial Evaluation

The activity of the synthetic compounds against the vulnerable bacteria *Euterococci, Escherichia coli, Staphylococcus aureus, Klebsiella spp.,* and *Proteusspp*., as well as two species of fungi, *Aspergillus niger and Candida albicans,* was assessed using the standard nutrient agar disc diffusion method [26-28]. 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 [27] classifies inhibition zones for tetracycline and fluconazole as resistant if they are greater than 14 mm, weakly sensitive if they are between 15 and 18 mm, and sensitive if they are greater than 19 mm. The findings also revealed that the investigated drugs' levels of inhibition differed (Table 1). The activity against both bacteria and fungus was significantly enhanced by the addition of the N-butyryl moiety. Future medicinal chemists may use the results of the current work to develop and create molecules with a similar structure with greater biological efficacy.

Table. Antimicrobial screening results of the tested compounds 4a-z.

Cpd. No.	Diameter of the inhibition zone in mm*						
	Antibacterial activity					Antifungal activity	
	Eutero-cocci	*Escheri-chiacoli*	*Staphylo-aureus*	*Klebsiellaspp*	*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]	23	20	22	21	23	--	--
Flu.[b]	--	--	--	--	--	26	25

DMSO	--	--	--	--	--	--	--

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

IV.CONCLUSION

New series of novel functionalized 1,3,4-oxadiazolines **4a-s** were synthesized upon the treatment of carbamoylhydrazones of aldehydes **3a-e**, ketones **3f-l** or cyclic ketones **3m-**s with butyric 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 butanoyl group at position-3 and at amino 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 antibacterial 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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