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Synthesis and biological activities of substituted 1,3,4-oxadiazolines

Hany M. Dalloul * and Kholoud R. Abu Yunis

Department of Chemistry, Faculty of Applied Sciences, Al-Aqsa University of Gaza, P.O.Box 4051, Gaza, Gaza Strip, 76888, Palestine

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Abstract: A new series of N-butyryl-1,3,4-oxadiazolines were synthesized *via* oxidative cyclization reaction of different benzoyl hydrazones with butyric 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 (*Euterococci*, *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: 1,3,4-Oxadiazolines; N-acylhydrazones; spiro-oxadiazole; oxidative cyclization.

1. Introduction

Oxadiazoles are important class of heterocyclic compounds. They are a five membered heterocyclic ring containing an oxygen atom and two nitrogen atoms with two double bonds, to give an aromatic ring having molecular formula $C_2H_2N_2O$. Oxadiazoles can be found in four different isomeric structures, namely; 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole. However, 1,3,4-oxadiazole and 1,2,4-oxadiazole are better known, and more widely studied by researchers[1-3]. From the literatures it's found that 1,3,4-oxadiazole and 1,2,4-oxadiazoles and their derivatives show more significant medicinal activity[4]. They are present in many drugs and biologically active compounds. These derivatives show variety of biological activity like antiviral, anticancer, anti-tubercular, anxiolytic, anti-inflammatory and many more[5].

Examples of drugs molecules containing oxadiazole heterocycle (Figure). Oxolamine is used as cough suppressant, Ataluren is a novel drug developed to treat Duchenne muscular dystrophy (DMD), and Furamizole is a strong antibacterial agent[6-8] cause irreversible damage to the cell membranes and, could oppose the release of the genetic material once in the nucleus. Raltegravir is an anti-viral drug, used for HIV infection[9], Nesapidil is a drug used in anti-arrhythmic therapy[10], Fasiplon is a 1,2,4-oxadiazole derivatives it is a non-benzodiazepine anxiolytic drug[10,11]. Tiodazosin is an alpha 1 adrenergic antagonists used as an anti-hypertensive agent[10,12] and Zibotentan is a new anti-cancer drug, especially used in prostate cancer[12,13]. Recently various Green synthetic procedures (microwave assisted method, grinding method, green catalysts assisted methods, electro-chemical method and ultra sound assisted method) of biologically active oxadiazole, derivatives have been reviewed[14-15].

* Corresponding author: hmdalloul@yahoo.com

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In current paper, we are attempting to construct a novel spirocycles containing oxadiazole moiety *via* treatment of benzoyl hydrazones with butyric anhydride.

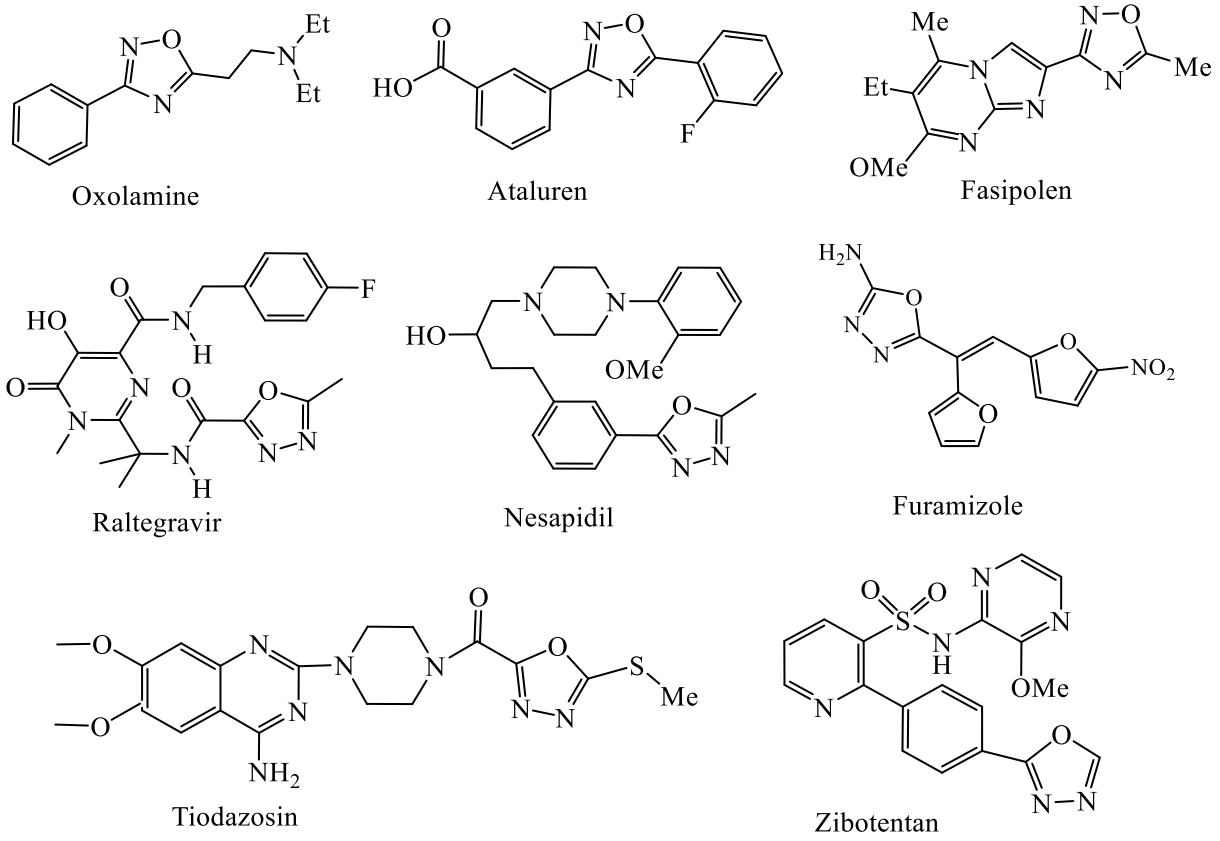


Figure 1. Examples of drugs molecules containing oxadiazole heterocycle

2. Experimental

2.1. Chemical Material and Apparatus

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) delivered by 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 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.

2.2. Chemistry

*2.2.1. General Procedure for Synthesis of acylhydrazones **3a-z***

A mixture of benzoic acid hydrazide **1** (0.01 mol) and aldehydes or ketones **2a-z** (0.01 mol) in ethanol (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 hydrazones **3a-z** in 70-85% yield[16-18].

*2.2.2. General Procedure for Synthesis of 1,3,4-oxadiazolines **4a-z***

A mixture of benzoyl hydrazones **3a-z** (10 mmol) and butyric anhydride (10-15 mL) was stirred under reflux in oil bath at 120-140 °C for 3-4 hrs. After the reaction was completed, the reaction mixture was allowed to cool, and then, the excess anhydride was removed *invacuo*, or the reaction mixture was left for 5-7 days at room temperature until dryness then the residue washed with potassium bicarbonate solution (10%) and finally with water and dried. The resulting crude solid product was then collected and recrystallized from aqueous ethanol (70%) in some cases from ethyl acetate to give the corresponding 1,3,4-oxadiazolines **4a-z**.

*1-(2-methyl-5-phenyl-1,3,4-oxadiazol-3(2H)-yl)butan-1-one (**4a**)*: White solid (58% isolated yield), m.p. 177-179 °C. FTIR (KBr, cm^{-1}): 2989 (C-H arom.), 1665 (C=O), 1566 (C=N), 1165 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.98 (t, 3H, *J* = 6.0 Hz, CH_3), 1.58 (d, 3H, *J* = 5.0 Hz, CH_3 of C_2-oxad.), 1.61 (m, 2H, CH_2), 2.31 (m, 2H, $COCH_2$), 6.50 (q, 1H, *J* = 5.4 Hz, CH of C_2-oxad.), 7.09-7.94 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.1 (CH_3 of butyryl), 18.8 (CH_2 of butyryl), 24.6 (CH_3), 48.9 (C=OCH_2 of butyryl), 89.7 (C_2-oxad.), 133.7, 131.7, 128.6, 127.3 (4 arom. C), 146.9 (C=N, oxadiazole ring), 166.9 (C=O). MS: *m/z* 232 [$M^{+\cdot}$]; Anal. Cald. for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06%. Found: C, 67.00; H 7.76; N, 12.25%.

*1-(2,5-diphenyl-1,3,4-oxadiazol-3(2H)-yl)butan-1-one (**4b**)*: White solid (68% isolated yield), m.p. 212-214 °C. FTIR (KBr, cm^{-1}): 3024 (C-H arom.), 1671 (C=O), 1606 (C=N), 1165 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.11 (s, 1H, CH at C_2-oxad.), 7.28-7.96 (m, 10H, arom.), ^{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), 91.8 (C_2-oxad.), 133.7, 132.9. 131.7, 131.5, 128.6, 128.4, 127.3, 127.2 (8 arom. C), 146.9 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 294 [M^+]; Anal. Cald. for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52%. Found; C, 73.71; H 5.94; N, 9.31%.

*1-[2-(2-hydroxyphenyl)-5-phenyl-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4c**)*: White solid (62% isolated yield), m.p. 156-158 °C. FTIR (KBr, cm^{-1}): 3255 (OH), 3022 (C-H arom.), 1659 (C=O), 1565 (C=N), 1165 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 5.32 (s, 1H, OH), 7.10 (s, 1H, CH at C_2-oxad.), 7.28-7.96 (m, 9H, arom.). ^{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), 91.7 (C_2-oxad.), 139.7, 133.6, 129.2, 128.6, 128.1, 127.3, 125.2 (9 arom. C), 146.9 (C=N, oxad.), 158.1 (C-OH), 166.9 (C=O).MS: *m/z* 310 [$M^{+\cdot}$]; Anal. Cald. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03%. Found; C, 69.96; H 6.07; N, 8.82%.

*1-[2-(furan-2-yl)-5-phenyl-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4d**)*: White solid (58% isolated yield), m.p. 217-219 °C. FTIR (KBr, cm^{-1}): 3096 (C-H arom.), 1657 (C=O), 1562 (C=N), 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 6.95 (s, 1H, CH at C_2-oxad.), 7.06-8.27 (m, 8H, arom.). ^{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.), 133.7, 131.5, 128.4, 127.2 (4 arom. C), 145.7, 139.4, 114.2, 112.8 (4 C furan ring), 146.9 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 284 [M^+]; Anal. Cald. for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.85%. Found; C, 67.30; H 6.89; N, 9.64%.

Synthesis of substituted 1,3,4-oxadiazolines

*1-[5-phenyl-2-(thiophen-2-yl)-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4e**)*: White solid (55% isolated yield), m.p. 213-215 °C. FTIR (KBr, cm^{-1}): 3084 (C-H arom.), 1659 (C=O), 1565 (C=N), 1162 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 6.98 (s, 1H, CH at C_2-oxad.), 7.12-8.27 (m, 8H, arom.). ^{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), 92.4 (C_2-oxad.), 133.7, 131.5, 128.4, 127.2 (4 arom. C), 144.8, 140.4, 119.6, 114.2 (4 C thiophene ring), 146.9 (C=N, oxad.), 166.9 (C=O).MS: *m/z* 300 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33%. Found; C, 64.25; H 5.15; N, 9.54%.

*1-(2-ethyl-2-methyl-5-phenyl-1,3,4-oxadiazol-3(2H)-yl)butan-1-one (**4f**)*: Pale yellow solid (58% isolated yield), m.p. 187-189 °C. FTIR (KBr, cm^{-1}): 3034 (C-H arom.), 1669 (C=O), 1556 (C=N), 1168 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.00 (t, 3H, *J* = 6.0 Hz, CH_3), 1.24 (t, 3H, *J* = 7.5 Hz, CH_3 of ethyl), 1.55 (s, 3H, CH_3 at C_2-oxad.), 1.63 (m, 2H, CH_2), 2.35 (m, 2H, $COCH_2$), 2.68 (d, 2H, *J* = 8.0 Hz, CH_2 of ethyl), 7.18-7.87 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 13.3 (CH_3 of ethyl), 18.9 (CH_2 of butyryl), 21.3 (CH_2 at C_2-oxad.), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.3 (quaternary C_2-oxad.), 133.7, 131.5, 128.4, 127.2 (4 arom. C), 153.8 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 260 [$M^{+\cdot}$]; Anal. Cald. for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76%. Found; C, 69.46; H 7.51; N, 10.98%.

*1-(2-cyclopropyl-2-methyl-5-phenyl-1,3,4-oxadiazol-3(2H)-yl)butan-1-one (**4g**)*: Yellow solid (68% isolated yield), m.p. 167-169 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1657 (C=O) 1561 (C=N), 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.52-0.67 (m, 4H, $2CH_2$ of cyclopropyl), 1.00 (t, 3H, *J* = 6.0 Hz, CH_3), 1.26-1.32 (m, 1H, CH of cyclopropyl), 1.56 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.36 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.18-7.92 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 13.3 (CH_3 of ethyl), 18.9 (CH_2 of butyryl), 21.3 (CH_2 at C_2-oxad.), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.1 (quaternary C_2-oxad.), 133.7, 131.5, 128.4, 127.2 (4 arom. C), 153.9 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 272 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29%. Found; C, 70.85; H 7.18; N, 10.52%.

*1-(2-methyl-2,5-diphenyl-1,3,4-oxadiazol-3(2H)-yl)butan-1-one (**4h**)*: White solid (59% isolated yield), m.p. 216-218 °C. FTIR (KBr, cm^{-1}): 3036 (C-H arom.), 1659 (C=O), 1564 (C=N), 1170 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.53 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, COCH2), 7.24-7.97 (m, 10H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.0 (quaternary C_2-oxad.), 139.2, 133.7, 131.5, 129.6, 128.4, 128.0, 127.2, 125.8 (8 arom. C), 146.9 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 308 [$M^{+\cdot}$]; Anal. Cald. for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08%. Found; C, 73.74; H 6.32; N, 8.87%.

*1-[2-(furan-3-yl)-2-methyl-5-phenyl-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4i**)*: Pale brown solid (58% isolated yield), m.p. 198-200 °C. FTIR (KBr, cm^{-1}): 3084 (C-H arom.), 1654 (C=O) 1556 (C=N) 1160 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.54 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.54-7.87 (m, 8H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.3 (quaternary C_2-oxad.), 131.6, 129.2, 125.6, 123.6, (4 arom. C), 130.3, 129.8, 128.2, 124.8 (4 C furan ring), 147.1 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 298 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39%. Found; C, 68.21; H 6.30; N, 9.15%.

*1-[2-methyl-5-phenyl-2-(thiophen-2-yl)-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4j**)*: Pale yellow solid (62% isolated yield), m.p. 177-179 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1658 (C=O), 1559 (C=N), 1161 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.56 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.48-7.97 (m, 8H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.4 (quaternary C_2-oxad.), 131.8, 129.5, 126.9, 125.2, (4 arom. C), 130.7, 130.1, 128.0,

125.8 (4 C thiophene ring), 146.5 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 314 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91%. Found; C, 65.15; H 5.56; N, 9.13%.

*1-[2-methyl-2-(naphthalen-2-yl)-5-phenyl-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4k**)*: Off-white solid (60% isolated yield), m.p. 227-229 °C. FTIR (KBr, cm^{-1}): 3024 (C-H arom.), 1645 (C=O), 1566 (C=N), 1245 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.53 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.34-8.57 (m, 12H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.6 (quaternary C_2-oxad.), 134.9, 133.7, 131.6, 130.3, 129.6, 128.4, 128.0, 127.2, 126.9, 126.4, 125.8, 125.2, 123.7, 121.8 (14 arom. C), 154.6 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 358 [$M^{+\cdot}$]; Anal. Cald. for $C_{23}H_2N_2O_2$: C, 77.07; H, 6.19; N, 7.82%. Found; C, 76.86; H 5.97; N, 8.06%.

*1-[2-(4-bromophenyl)-2-methyl-5-phenyl-1,3,4-oxadiazol-3(2H)-yl]butan-1-one (**4l**):* White solid (66% isolated yield), m.p. 197-199 °C. FTIR (KBr, cm^{-1}): 2098 (C-H), 1661 (C=O), 1566 (C=N), 1165 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.51 (s, 3H, CH_3 of C_2 oxad.), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.24-7.87 (m, 9H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 100.2 (quaternary C_2-oxad.), 133.7, 132.7, 128.5, 128.0, 126.6, 127.2, 123.8, 122.9 (8 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 387 [$M^{+\cdot}$]; Anal. Cald. for $C_{19}H_{19}BrN_2O_2$: C, 58.93; H, 4.95; N, 7.23%. Found; C, 59.20; H 4.72; N, 7.45%.

*1-(3-Phenyl-4-oxa-1,2-diazaspiro[4.4]non-2-en-1-yl)butan-1-one (**4m**)*: White solid (68% isolated yield), m.p. 201-203 °C. FTIR (KBr, cm^{-1}): 3038 (C-H arom.), 1665 (C=O), 1556 (C=N), 1245 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.66-1.86 (m, 8H, 4CH_2 cyclopentane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.21-7.97 (m, 5H, arom.). ^{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), 38.4, 36.1, 24.4, 23.2 (4CH_2 cyclopentane), 100.1 (spiro C_2-oxad.), 131.5, 129.6, 128.1, 126.8 (4 arom. C), 154.6 (C=N, oxad.), 166.9 (C=O). MS: *m/z* 272 [M^+]; Anal. Cald. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29%. Found; C, 70.81; H 7.18; N, 10.07%.

*1-(3-Phenyl-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)butan-1-one (**4n**)*: Off-white solid (64% isolated yield), m.p. 211-213 °C. FTIR (KBr, cm^{-1}): 3042 (C-H arom.), 1663 (C=O), 1559 (C=N), 1249 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.56-2.11 (m, 10H, 5CH_2 cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.19-7.93 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 33.0, 31.4, 24.8, 23.4, 21.6 (5CH_2 cyclohexane), 100.2 (spiro C_2-oxad.), 132.6, 131.6, 128.4, 127.2, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 286 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78%. Found; C, 71.51; H 7.56; N, 9.55%.

*1-(3-Phenyl-4-oxa-1,2-diazaspiro[4.6]undec-2-en-1-yl)butan-1-one (**4o**)*: White solid (68% isolated yield), m.p. 188-192 °C. FTIR (KBr, cm^{-1}): 3048 (C-H arom.), 1665 (C=O), 1558 (C=N) 1248 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.60-2.31 (m, 12H, 6CH_2 cycloheptane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.21-7.96 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 35.2, 32.7, 30.1, 21.7, 21.6, 13.5 (6CH_2 cycloheptane), 100.3 (spiro C_2-oxad.), 133.2, 131.9, 128.7, 126.8, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 300 [$M^{+\cdot}$]; Anal. Cald. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33%. Found; C, 71.75; H 7.83; N, 9.57%.

*1-(3-Phenyl-4-oxa-1,2-diazaspiro[4.7]dodec-2-en-1-yl)butan-1-one (**4p**)*: White solid (56% isolated yield), m.p. 167-169 °C. FTIR (KBr, cm^{-1}): 3048 (C-H arom.), 1668 (C=O), 1557 (C=N), 1249 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.44-2.26 (m, 14H, 7CH_2 cyclooctane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.23-7.98 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 37.8, 29.1, 28.8, 26.8, 26.6, 22.3, 22.1 (7CH_2 cyclooctane), 100.1 (spiro C_2-oxad.), 134.3, 131.8,

128.9, 127.1, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 314 [$M^{+\cdot}$]; Anal. Cald. for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91%. Found; C, 72.87; H 8.05; N, 9.18%.

1-(8-Methyl-3-phenyl-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)butan-1-one (***4q***): White solid (72% isolated yield), m.p. 198-201 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1668 (C=O), 1560 (C=N) 1246 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.95 (d, 3H, *J* = 3.5 Hz, C-CH_3), 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.56-2.11 (m, 10H, 5CH_2 cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.19-7.96 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 24.6 (CH_3 at C_2-oxad.), 48.8 (C=OCH_2 of butyryl), 31.4 (CH_3 at cyclohexane), 33.1, 28.7, 24.8, 24.4 (4CH_2 cyclohexane), 38.1 (CH of cyclohexane), 98.9 (spiro C_2-oxad.), 133.0, 130.8, 128.7, 127.6, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 300 [$M^{+\cdot}$]; Anal. Cald. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33%. Found; C, 72.25; H 7.83; N, 9.61%.

1-(8-t-Butyl-3-phenyl-4-oxa-1,2-diazaspiro[4.5]dec-2-en-1-yl)butan-1-one (***4r***): White solid (70% isolated yield), m.p. 178-181 °C. FTIR (KBr, cm^{-1}): 3048 (C-H arom.), 1664 (C=O), 1561 (C=N), 1247 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.85 (s, 9H, 3CH_3 t-Bu), 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.10-2.11 (m, 9H, 4CH_2, CH cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.18-7.94 (m, 5H, arom.). ^{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), 38.1, (CH_3 at cyclohexane), 33.1, 28.7, 24.8, 24.4 (4CH_2 cyclohexane), 38.1 (CH of cyclohexane), 99.7 (spiro C_2-oxad.), 132.6, 131.5, 128.6, 126.8, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 342 [$M^{+\cdot}$]; Anal. Cald. for $C_{21}H_{30}N_2O_2$: C, 73.65; H, 8.83; N, 8.18%. Found; C, 73.95; H 9.02; N, 7.98%.

1-(8-methyl-3-phenyl-4-oxa-1,2,8-triazaspiro[4.5]dec-2-en-1-yl)butan-1-one (***4s***): Pale yellow solid (78% isolated yield), m.p. 207-210 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1670 (C=O), 1564 (C=N), 1250 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.80-2.80 (m, 8H, 4CH_2 cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 2.41 (s, 3H, NCH_3), 7.16-7.89 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 31.1 (2CH_2), 46.7 (NCH_3), 48.8 (C=OCH_2 of butyryl), 53.2 (2CH_2), 100.8 (spiro C_2-oxad.), 134.6, 132.1, 127.9, 125.9 (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 300 [M^+]; Anal. Cald. for $C_{17}H_{23}N_3O_2$: C, 67.75; H, 7.69; N, 13.94%. Found; C, 67.98; H 7.49; N, 14.12%.

1-(8-Isopropyl-3-phenyl-4-oxa-1,2,8-triazaspiro[4.5]dec-2-en-1-yl)butan-1-one (***4t***): Off-white solid (73% isolated yield), m.p. 217-219 °C. FTIR (KBr, cm^{-1}): 3058 (C-H arom.), 1675 (C=O), 1565 (C=N), 1253 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.24 (s, 6H, 2CH_3 isopr.),1.63 (m, 2H, CH_2), 1.80-3.05 (m, 8H, 4CH_2 cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 2.40 (m, 1H, NCH),7.17-7.88 (m, 5H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 27.6 (2CH_3), 30.9 (2CH_2), 47.5 (NCH), 48.8 (C=OCH_2 of butyryl), 53.1 (2CH_2), 100.9 (spiro C_2-oxad.), 134.8, 131.9, 128.2, 127.0, (4 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 329 [$M^{+\cdot}$]; Anal. Cald. for $C_{19}H_{27}N_3O_2$: C, 69.27; H, 8.26; N, 12.75%. Found; C, 69.02; H 8.44; N, 12.57%.

1-(8-Benzyl-3-phenyl-4-oxa-1,2,8-triazaspiro[4.5]dec-2-en-1-yl)butan-1-one (***4u***): White solid (71% isolated yield), m.p. 169-172 °C. FTIR (KBr, cm^{-1}): 3060 (C-H arom.), 1672 (C=O), 1565 (C=N), 1255 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 1.70-2.82 (m, 8H, 4CH_2 cyclohexane), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 3.31 (s, 2H, NCH_2), 7.20-8.23 (m, 10H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 31.1 (2CH_2), 48.8 (C=OCH_2 of butyryl), 50.2 ($PhCH_2$), 53.2 (2CH_2), 101.2 (spiro C_2-oxad.), 140.9, 136.1, 133.6, 131.8, 128.4, 127.2, 125.8, 115.6 (8 arom. C), 154.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 377 [$M^{+\cdot}$]; Anal. Cald. for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13%. Found; C, 73.39; H 7.37; N, 11.01%.

1-(5'-phenyl-3'H-spiro[4-azabicyclo[2.2.2]octane-2,2'-[1,3,4]oxadiazol]-3'-yl)butan-1-one (***4v***):
White solid (36% isolated yield), m.p. 236 °C dec. FTIR (KBr, cm^{-1}): 3096 (C-H arom.), 1680 (C=O), 1612 (C=N), 1259 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m,

2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.19-8.26 (m, 13H, arom.).^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 25.8 ($2CH_2$), 33.7 (CH), 48.8 (C=OCH_2 of butyryl), 50.2 ($2CH_2$), 55.8 (CH_2), 102.6 (spiro C), 133.6, 131.2, 128.6, 127.3 (4 arom. C), 146.9 (C=N, oxad.), 158.1 (C-OH), 166.9 (C=O). MS: *m/z* 313 [M^+]; Anal. Cald. for $C_{18}H_{23}N_3O_2$: C, 68.98; H, 7.40; N, 13.41%. Found; C, 69.32; H 7.12; N, 13.75%.

1-(5'-phenyl-3,4-dihydro-3'H,9H-spiro[fluoren-9-yl-[1,3,4]oxadiazol]-3'-yl)butan-1-one (***4x***): Yellow solid (34% isolated yield), m.p. 226-229 °C. FTIR (KBr, cm^{-1}): 3076 (C-H arom.), 1668 (C=O), 1605 (C=N), 1256 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.02 (t, 3H, *J* = 6.0 Hz, CH_3), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.19-8.26 (m, 13H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 25.8 ($2CH_2$), 33.7 (CH), 48.8 (C=OCH_2 of butyryl), 50.2 ($2CH_2$), 55.8 (CH_2), 104.2 (spiro C), 133.6, 131.2, 128.6, 127.3 (4 arom. C), 146.9 (C=N, oxad.), 158.1 (C-OH), 166.9 (C=O). MS: *m/z* 368 [M^+]; Anal. Cald. for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60%. Found; C, 77.93; H 5.23; N, 7.32%.

1-(5'-phenyl-3,4-dihydro-2H,3'H-spiro[naphthalene-1,2'-[1,3,4]oxadiazol]-3'-yl)butan-1-one (***4y***): Orange solid (41% isolated yield), m.p. 199-202 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1652 (C=O), 1560 (C=N), 1253 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, 3H, *J* = 6.0 Hz, CH_3), 1.10-2.12 (m, 6H, $3CH_2$ tetralinyl), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.21-7.98 (m, 9H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 25.8 ($2CH_2$), 33.7 (CH), 48.8 (C=OCH_2 of butyryl), 50.2 ($2CH_2$), 55.8 (CH_2), 103.7 (spiro C), 133.6, 131.2, 128.6, 127.3 (4 arom. C), 146.9 (C=N, oxad.), 158.1 (C-OH), 166.9 (C=O). MS: *m/z* 334 [M^+]; Anal. Cald. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38%. Found; C, 75.68; H 6.41; N, 8.61%.

1-(5'-phenyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4]oxadiazol]-3'-yl)butan-1-one (***4z***): White solid (43% isolated yield), m.p. 217-220 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1650 (C=O), 1559 (C=N), 1249 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.01 (t, *J* = 6.0 Hz, 3H, CH_3), 1.10-2.12 (s, 4H, $2CH_2$ indanyl), 1.63 (m, 2H, CH_2), 2.33 (t, 2H, *J* = 6.0 Hz, $COCH_2$), 7.30-7.98 (m, 9H, arom.). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.0 (CH_3 of butyryl), 18.9 (CH_2 of butyryl), 36.7 ($2CH_2$), 48.8 (C=OCH_2 of butyryl), 105.3 (spiro C), 133.6, 131.2, 128.6, 127.3 (4 arom. C), 146.9 (C=N, oxad.), 158.1 (C-OH), 166.9 (C=O). MS: *m/z* 320 [M^+]; Anal. Cald. for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74%. Found; C, 75.32; H 6.52; N, 8.45%.

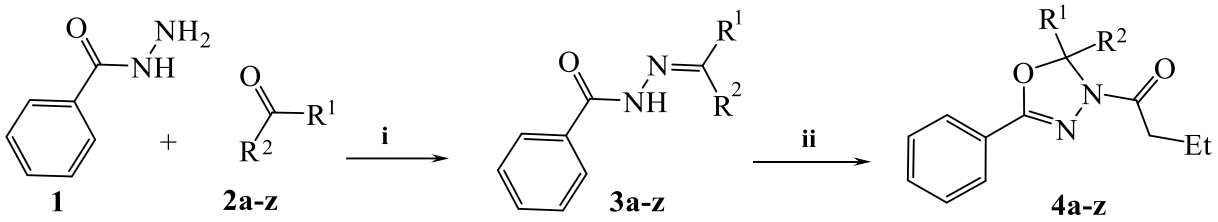
3. Results and Discussion

3.1. Chemistry

The precursors N-acylhydrazones employed, in this study, were prepared by condensation of benzoic acid hydrazide **1** with the corresponding carbonyl compounds **2a-z** (aldehydes or ketones) in refluxing ethanol producing benzoyl hydrazones **3a-z** (Scheme1) with yields ranging 80-95%. The treatment of hydrazones **3a-z** with refluxing excess butyric anhydride furnished a new series of 2,3-dihydro-1,3,4-oxadiazole derivatives **4a-z** (Scheme1) in good yields, after purification by recrystallization using ethanol or ethyl acetate. The purity of the compounds was checked by TLC and their elemental analysis, which matched within ±0.3-0.4 percentage of the theoretical values. The structures of the newly synthesized compounds were 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.

Cycloaddition is achieved by ring formation that results from intramolecular cyclization of hydrazones **3a-z** and deprotonation step forms 2,3-dihydro-1,3,4-oxadiazole derivatives **4a-z** as shown in Scheme 2. The structures of all the synthesized 1,3,4-oxadiazolines **4a-z** were confirmed by elemental analysis and spectroscopic methods. The spectroscopic studies proved the successful butyric anhydride-promoted oxidative cyclization of N-acylhydrazones **3a-z**.

Synthesis of substituted 1,3,4-oxadiazolines



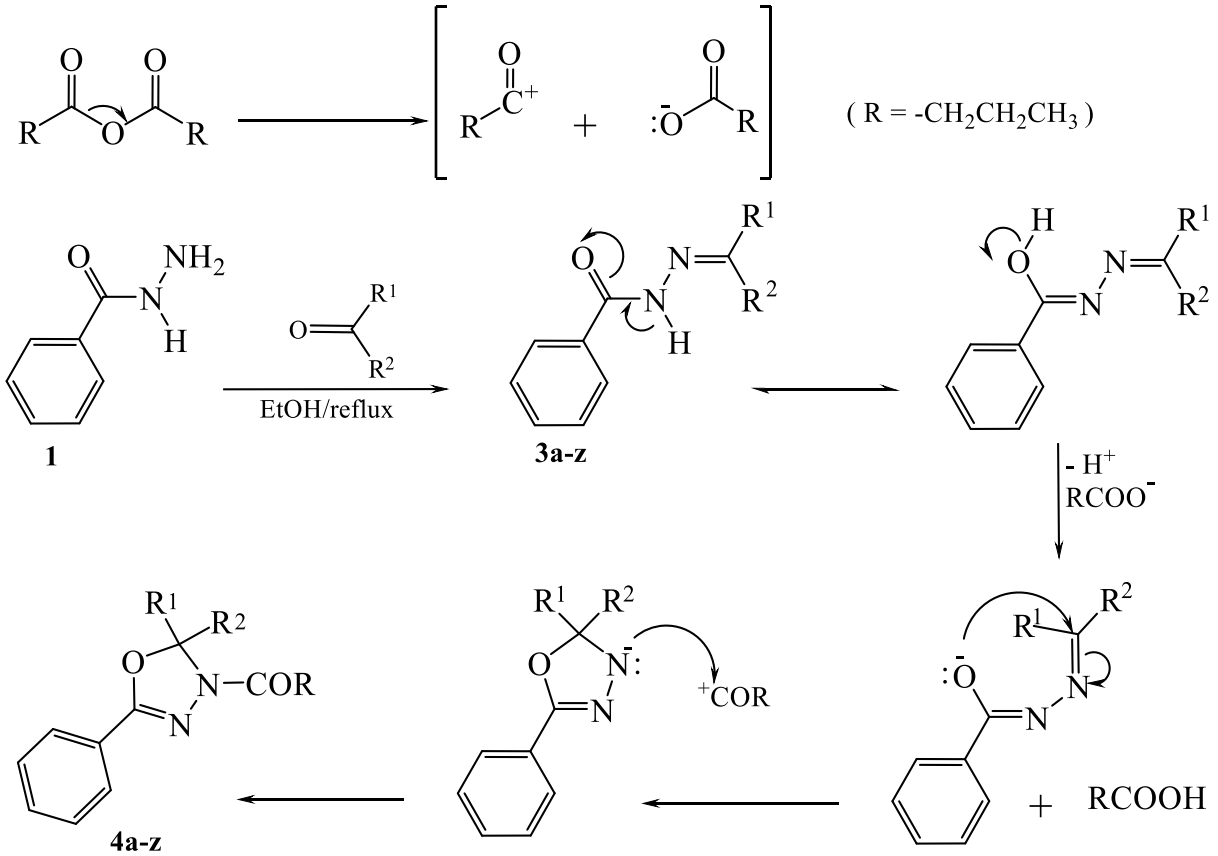
i) EtOH/reflux; **ii**) Butyric anhydride/reflux

R^1 / R^2 = **a:** H/Me; **b:** H/Ph; **c:** H/2-HOPh; **d:** H/2-Furyl; **e:** H/2-Thienyl; **f:** Me/Et; **g:** Me/cyclo-Pr; **h:** Me/Ph; **i:** Me/2-Furyl; **j:** Me/2-Thienyl; **k:** Me/2-Naphthyl; **l:** Me/4-BrPh; **m:** -$(CH_2)_4$-; **n:**-$(CH_2)_5$-; **o:**-$(CH_2)_6$-; **p:**-$(CH_2)_7$-; **q:**-$(CH_2)_2$-(CHMe)-$(CH_2)_2$-; **r:**-$(CH_2)_2$-CMe_3-$(CH_2)_2$-; **s:** -$(CH_2)_2$-(NMe)-$(CH_2)_2$-; **t:** -$(CH_2)_2$-N($CHMe_2$)-$(CH_2)_2$-; **u:** -$(CH_2)_2$-N(CH_2Ph)-$(CH_2)_2$-; **v:** 3-Quinuclidinyl; **x:** 9-Florenyl; **y:** 2-Tetralinyl; **z:** 2-Indanyl.

Scheme 1. Schematic synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives **4a-z**

*3.2. Spectral Data Analysis of Compounds **4a-z***

In the IR spectra of 1,3,4-oxadiazolines **4a-z** the characteristic peak for the amide NH at 3200-3250 cm^{-1} and C=O at 1630-1650 cm^{-1} in the starting acylhydrazones **3a-z** completely disappeared from the IR spectra of the obtained products **4a-z**. A new C=O stretching of the butyryl group appeared at 1660-1680 cm^{-1}, C-O-C of ring at 1160-1250 cm^{-1}, and C=N stretching of dihydro-oxadiazole ring appeared in the range of 1550-1610 cm^{-1}. The second confirmation of the correct structure for compounds **4a-z**, comes from their mass spectra were found in good agreement with the newly synthesized compounds.



Scheme 2. A plausible mechanism for the formation of 1,3,4-oxadiazolines **4a-z**

The ^{1}H-NMR spectra provided clear evidence about the right structure of synthesized compounds **4a-z**. The first evidence comes from the disappearance of the characteristic proton of the NH group at 10.0-11.1 ppm, in the ^{1}H-NMR spectra of starting hydrazones **3a-z**. The disappearance of the NH proton was accompanied by the appearance of a three signals at 2.5 (t), 1.7(m) and 0.91(t) ppm and these peaks were assigned to the protons ($CH_2CH_2CH_3$) of the butyryl group indicating the formation of the N-substituted oxadiazoline ring. In compounds, **4a-e** the O-CH-N proton was resonated in the range of 6.5-7.1 ppm instead of 8.5 ppm as appeared in precursor hydrazones **3a-e**. For compounds **4m-u** 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-z** there are a new peaks appeared for butyryl group carbons and this indeed are expected, because they are not part of starting hydrazones **3a-z**. Where the carbonyl (C=O) of the butyryl group appeared at 166.6 ppm and the other peaks appeared around 32.7, 16.6 and 13.1 ppm are assigned to be the peaks of the $CH_2CH_2CH_3$ of the butyryl group.

In the same spectra, two major new peaks appeared and both of them confirm the formation of the N-butyryl oxadiazoline ring. The first new and significant peak appeared at 90.0-92.0 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-z** 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[18,19]. 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-oxadiazole ring.

Table 1. Antimicrobial screening results of the tested compounds **4a-z**

Compd. No	Diameter of the inhibition zone in mm*						
	Antibacterial activity					Antifungal activity	
	Euterococci	***E. coli***	***S. aureus***	***Klebsiella spp***	***Proteus spp***	***C.albicans***	***A. niger***
4a	18	16	18	14	16	14	17
4b	14	18	15	15	13	19	16
4c	19	15	11	14	16	18	16
4d	13	16	17	18	19	16	13
4e	16	19	16	19	11	19	12
4f	17	12	14	16	17	16	19
4g	19	15	11	14	16	18	16
4h	18	16	17	18	19	16	12
4i	16	19	16	19	11	19	11
4j	13	12	14	16	17	16	19
4k	16	17	17	13	16	15	14
4l	13	18	15	11	10	17	18
4m	19	15	11	14	16	18	16
4n	16	16	17	18	19	16	12
4o	17	19	16	19	11	19	11
4p	15	12	14	16	17	16	19
4q	18	15	11	14	16	18	16
4r	17	16	17	18	19	16	12
4s	15	19	16	19	11	19	18
4t	13	12	14	16	17	14	19
4u	12	12	14	16	17	16	19
4v	17	15	11	14	16	18	16
4x	18	16	17	18	19	16	17
4y	16	19	16	19	11	19	11
4z	14	12	14	16	17	16	19
Tet.[a]	23	20	22	21	23	--	--
Flu.[b]	--	--	--	--	--	26	25
DMSO	--	--	--	--	--	--	--

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

3.3. Biological Activities

The activity of the synthetic compounds against the vulnerable bacteria *Euterococci*, *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[20,21]. 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[21] 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.

4. Conclusion

New series of novel functionalized 1,3,4-oxadiazolines **4a-z** were synthesized upon the treatment of benzoic acid hydrazones of aldehydes **3a-e**, ketones **3f-l** or cyclic ketones **3m-z** 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 butyryl group at position-3 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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ORCID
Hany M. Dalloul: 0000-0002-4947-3648
Kholoud Abu Yunis: 0000-0001-8122-6332

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