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Synthesis and Biological Activities Evaluation of Novel Spiroheterocycles Containing 1,3,4-Oxadiazoline Moiety

Hany M. Dalloul and Kholoud R. Abu-Yunis

ABSTRACT

A novel series of spiro 3-acetyl-1,3,4-oxadiazolines were synthesized *via* oxidative cyclization reaction of different 2-furoyl and 2-thenoyl hydrazones 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 newly synthesized spiro 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: 2-Furoylhydrazone, 2-Thenoyl-Hydrazone, Oxadiazole, Oxidative Cyclization, Spiro-1,3,4-Oxadiazoline.

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I. Introduction

There has been an endless “race” between scientists developing new drugs and pathogenic bacteria since introducing the penicillin in 1942, as the first antibiotic into clinical medical practice. The prevalence of antibiotic-resistant bacteria has increased, making matters worse and increasing the number of bacteria immune to all known antibiotics. Introduction of new antibiotics that kill resistance mutants is the traditional approach to solve this problem. Exactly the "arms race" that has led to the development of thousands of potentially useful chemicals in laboratories around the world every day. The synthesis of nitrogen- and oxygen-containing scaffolds has gained popularity due to its versatility in the drug discovery arsenal. Oxadiazole is a bioisomer of (hydroxamic) esters, carboxamides, and carbamates, which makes it more stable than the original scaffold. Oxadiazole is particularly useful in the pharmaceutical industry for drug discovery, luminescent materials, and dye manufacturing [1]-[5].

Now a day there are many commercially available drugs that contain the nucleolus oxadiazole, such as Placonaryl (an anti-infective), Oxolamine (an anti-inflammatory), Prenoxdiazine (a cough suppressant), Butalamine (a vasodilator), Ataloren (used in duchenne muscular dystrophy), and Bruxazole (used as an antitussive and anti-plasma). Some marine natural products, such as Phidianidines, also possess 1,2,4-oxadiazole as major pharmacological features. In addition, 1,3,4-oxadiazole has been identified as an important scaffold of pharmaceutical uses [6],[7], Zibotentan is an anticancer drug produced by Astra Zeneca that has been FDA approved for the treatment of prostate cancer [8]. The FDA also approved Raltegravir for Merck in conjunction with other antiretroviral agents for the treatment of HIV-1 infection [9]. Furamizole, a powerful antibacterial antibiotic, and Nesapidil, an antihypertensive agent [8].

1,3,4-Oxadiazole and their derivatives are involved in modifications such as attaching a carbon at position 2 and 5 with various substitutions, which often residuals of the synthetic starting materials such as simple aliphatic groups, substituted aliphatic chains, aromatic carbocyclic and heterocyclic rings [10]. The primary pathways to the 1,3,4-oxadiazole skeleton involve oxidative cyclization of N-acylhydrazones with a series of oxidants including molecular iodine, 2,3-dichloro-5,6-dicyanobenzoquinone, iodobenzene diacetate, and others. Another simple method is to use microwave irradiation to enhance the cyclocondensation of 2-acyl-N-phenylhydrazine carboxamides in presence of phosphorus oxychloride [11].

Moreover, synthesis of various biologically active oxadiazole derivatives applying green chemistry procedures such as, grinding method, green catalysts assisted methods, electro-chemical method and ultrasound assisted method have been reviewed [12].

Spiro compounds have attracted particular interest in medicinal chemistry due to the presence of spiro carbon, which gives the structure rigidity and allows adjustment along precise vectors. Because of their

distinctive conformational properties, structural complexity, and rigidity, spiro compounds-two cyclic rings fused to a single carbon atom-represent interesting structural systems in the field of drug discovery [13]. An efficient route for the synthesis of five-membered spiro compounds is 1,3-dipolar cycloaddition [14]. Although 1,3-dipole interactions with olefinic substrates have been extensively studied [15], [16], but the methods for oxadiazole synthesis using 1,3-dipolar reactions with carbonyl units are uncommon [17].

According to the preceding facts, and in continuation of our attention in the synthesis of pharmacologically and chemically useful compounds, it seemed a great idea to prepare various spiro heterocyclic compounds bearing 1,3,4-oxadiazole moieties and investigate their biological activities. So here in this research we outline the synthesis of a novel spiroheterocycles containing 1,3,4-oxadiazole moieties by the reaction of different 2-furoyl and 2-thenoyl hydrazones with acetic anhydride.

II. Experimental Section

A. Material 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 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 (Belgium) 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. (Germany).

B. Chemical Synthesis

1) General Procedure for Synthesis of acylhydrazones 3a-z

A mixture of 2-furoyl or 2-thenoyl hydrazine 1a-h (0.01 mol) and ketones 2a-h (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-p in 80-90% yield [18], [19].

2) General Procedure for Synthesis of spiro-1,3,4-oxadiazolines 4a-z

The 0.01mole of appropriate hydrazide-hydrazone of acid hydrazide 3a-h and excess acetic anhydride (15-20 mL) was heated under reflux in oil bath at 120-140 ºC for 2-4 hrs. After the reaction was completed, the reaction mixture was allowed to cool, and then, the excess anhydride was removed under reduced pressure, and the remains material poured into crushed ice with vigorous shaking for 10 min, then left at room temperature for 24h. The resulting crude solid product was filtered off and recrystallized from ethanol/ethyl acetate (3:1 v/v) mixture to afford the corresponding 1,3,4-oxadiazolines 4a-p.

1-Acetyl-3-(2-furyl)-8-methyl-4-oxa-1,2-diazaspiro[4.5]dec-2-ene (4a): White solid (77% yield), Mp: 172-174 °C. FTIR (KBr, cm^{-1}): 3089 (C-H arom.), 1669 (C=O), 1516 (C=N), 1241 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 0.95 (d, 3H, C-CH_3), 1.50-2.18 (m, 9H, 4CH_2+CH cyclohexane), 2.32 (s, 3H, $COCH_3$), 6.52-7.56 (m, 3H, furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.8 (CH_3), 31.4 (CH_3 at cyclohexane), 33.1, 28.7, 24.8, 24.4 (4CH_2 cyclohexane), 38.1 (CH of cyclohexane), 102.3 (spiro C_2-oxad.), 133.0, 130.8, 128.7, 127.6, (4 furan C), 151.9 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 262 [$M^{+\cdot}$]; Anal. Cald. for $C_{14}H_{18}N_2O_3$: C, 64.11; H, 6.92; N, 10.68%. Found: C, 64.42; H 7.08; N, 10.50%.

1-Acetyl-8-t-butyl-3-(2-furyl)-4-oxa-1,2-diazaspiro[4.5]dec-2-ene (4b): White solid (76% yield), Mp: 181-183 °C. FTIR (KBr, cm^{-1}): 3048 (C-H arom.), 1669 (C=O), 1521 (C=N), 1257 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 0.86 (s, 9H, 3CH_3 t-Bu), 1.30-2.14 (m, 9H, 4CH_2+ CH cyclohexane), 2.36 (s, 3H, $COCH_3$), 6.53-7.54 (m, 3H, furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.6 (CH_3), 33.1, 28.7, 24.8, 24.4 (4CH_2 cyclohexane), 38.0 (CH of cyclohexane), 103.7 (spiro C_2-oxad.), 132.6, 131.5, 128.6, 126.8, (4 furan C), 151.9 (C=N, oxad.), 167.2 (C=O). MS: *m/z* 304 [M^{+}]; Anal. Cald. for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20%. Found; C, 66.82; H 8.12; N, 9.02%.

1-Acetyl-3-(2-furyl)-8-methyl-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4c): Pale yellow solid (78% isolated yield), Mp: 207-210 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1672 (C=O), 1514 (C=N), 1254 (C-

O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.82-2.80 (m, 8H, 4CH_2 cyclohexane), 2.31 (s, 3H, $COCH_3$), 2.45 (s, 3H, NCH_3), 6.56-7.59 (m, 3H, furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.3 (CH_3), 34.6 (2CH_2), 46.7 (NCH_3), 53.2 (2CH_2), 103.4 (spiro C_2-oxad.), 134.6, 132.1, 127.9, 125.9 (4 furan C), 152.6 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 263 [$M^{+\cdot}$]; Anal. Cald. for $C_{13}H_{17}N_3O_3$: C, 59.30; H, 6.51; N, 15.96%. Found; C, 59.63; H 6.69; N, 16.12%.

1-Acetyl-3-(2-furyl)-8-isopropyl-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4d): Off-white solid (73% yield), Mp: 167-169 °C. FTIR (KBr, cm^{-1}): 3058 (C-H arom.), 1673 (C=O), 1515 (C=N), 1251 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.27 (d, 6H, 2CH_3 isopropyl), 1.87-3.02 (m, 8H, 4CH_2 cyclohexane), 2.33 (s, 3H, $COCH_3$), 2.48 (m, 1H, NCH), 6.60-7.58 (m, 3H, furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.8 (CH_3), 27.6 (2CH_3), 34.4 (2CH_2), 47.5 (NCH), 53.1 (2CH_2), 101.9 (spiro C_2-oxad.), 134.8, 131.9, 128.2, 127.0, (4 furan C), 152.8 (C=N, oxad.), 167.3 (C=O). MS: *m/z* 291 [M^{+}]; Anal. Cald. for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27; N, 14.42%. Found; C, 61.56; H 7.10; N, 14.60%.

1-Acetyl-8-benzyl-3-(2-furyl)-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4e): White solid (76% yield), Mp: 165-167 °C. FTIR (KBr, cm^{-1}): 3060 (C-H arom.), 1672 (C=O), 1519 (C=N), 1252 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.70-2.82 (m, 8H, 4CH_2 cyclohexane), 2.33 (s, 3H, CH_3), 3.34 (s, 2H, NCH_2), 6.58-7.95 (m, 7H, arom. + furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.8 (CH_3), 34.5 (2CH_2), 50.2 ($PhCH_2$), 53.2 (2CH_2), 105.1 (spiro C_2-oxad.), 140.9, 136.1, 133.6, 131.8, 128.4, 127.2, 125.8, 115.6 (8 arom. + furan C), 152.9 (C=N, oxad.), 167.9 (C=O). MS: *m/z* 339 [$M^{+\cdot}$]; Anal. Cald. for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38%. Found; C, 67.53; H 6.41; N, 12.15%.

3'-Acetyl-5'-(2-furyl)-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4]-oxadiazole] (4f): Pale yellow solid (65% yield), m.p. 217-220 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1665 (C=O), 1521 (C=N), 1228 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.16-2.21 (s, 4H, 2CH_2 indanyl), 2.33 (s, 3H, $COCH_3$), 6.59-7.98 (m, 7H, arom.+ furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 36.7 (2CH_2), 23.1 (CH_3), 105.3 (spiro C), 133.6, 131.2, 128.6, 127.3 (7 arom.+ furan C), 151.9 (C=N, oxad.), 168.2 (C=O). MS: *m/z* 282 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{14}N_2O_3$: C, 68.08; H, 5.00; N, 9.92%. Found; C, 67.76; H 4.81; N, 10.03%.

3'-Acetyl-5'-(2-furyl)-3,4-dihydro-2H,3'H-spiro[naphthalene-1,2'-[1,3,4]-oxadiazole] (4g): Orange solid (63% yield), Mp: 201-203 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1668 (C=O), 1520 (C=N), 1253 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.14-2.26 (m, 6H, 3CH_2 tetralinyl), 2.36 (s, 3H, $COCH_3$), 6.61-8.08 (m, 7H, arom.+ furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 23.4 ($C=OCH_3$), 25.8, 34.2 (2CH_2), 50.2 (CH_2), 103.7 (spiro C), 133.6, 131.2, 128.6, 127.3 (6 arom.+ furan C), 151.7 (C=N, oxad.) 168.9 (C=O). MS: *m/z* 296 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45%. Found; C, 68.57; H 5.65; N, 9.23%.

3'-Acetyl-5'-(2-furyl)-3,4-dihydro-3'H,9H-spiro[9-fluorene-[1,3,4]oxadiazole] (4h): Yellow solid (62% yield), Mp: 226-229 °C. FTIR (KBr, cm^{-1}): 3076 (C-H arom.), 1672 (C=O), 1605 (C=N), 1256 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 2.39 (s, 3H, $COCH_3$), 6.62-8.12 (m, 11H, arom.+furan). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 24.1 (CH_3), 50.2 (2CH_2), 104.2 (spiro C), 133.6, 131.2, 128.6, 127.3 (10 arom. .+furan C), 152.3 (C=N, oxad.), 168.5 (C=O). MS: *m/z* 330 [$M^{+\cdot}$]; Anal. Cald. for $C_{20}H_{14}N_2O_3$: C, 72.72; H, 4.27; N, 8.48%. Found; C, 73.06; H 4.51; N, 8.69%.

1-Acetyl-8-methyl-3-(2-thienyl)-4-oxa-1,2-diazaspiro[4.5]dec-2-ene (4i): Pale brown solid (78% yield), Mp: 198-200 °C. FTIR (KBr, cm^{-1}): 3089 (C-H arom.), 1665 (C=O), 1519 (C=N), 1228 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 0.95 (d, 3H, C-CH_3), 1.47-2.16 (m, 9H, 4CH_2+CH cyclohexane), 2.34 (s, 3H, $COCH_3$), 6.50-7.52 (m, 3H, thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.4 (CH_3), 31.6 (CH_3 at cyclohexane), 33.3, 28.6, 24.7, 24.3 (4CH_2 cyclohexane), 38.2 (CH of cyclohexane), 103.4 (spiro C_2-oxad.), 133.0, 130.8, 128.7, 127.6, (4 thiophene C), 152.4 (C=N, oxad.), 167.6 (C=O). MS: *m/z* 278 [$M^{+\cdot}$]; Anal. Cald. for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.92; N, 9.51%. Found; C, 60.70; H 7.10; N, 9.33%.

1-Acetyl-8-t-butyl-3-(2-thienyl)-4-oxa-1,2-diazaspiro[4.5]dec-2-ene (4j): Pale yellow solid (72% yield), Mp: 177-179 °C. FTIR (KBr, cm^{-1}): 3048 (C-H arom.), 1667 (C=O), 1520 (C=N), 1227 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 0.89 (s, 9H, 3CH_3 t-Bu), 1.33-2.17 (m, 9H, 4CH_2+ CH cyclohexane), 2.36 (s, 3H, $COCH_3$), 6.51-7.53 (m, 3H, thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.7 (CH_3), 33.7, 28.5, 24.6, 24.2 (4CH_2 cyclohexane), 38.3 (CH of cyclohexane), 104.1 (spiro C_2-oxad.), 132.6, 131.5, 128.6, 126.8, (4 thiophene C), 152.2 (C=N, oxad.), 167.9 (C=O). MS: *m/z* 320 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74%. Found; C, 63.41; H 7.73; N, 8.56%.

1-Acetyl-8-methyl-3-(2-thienyl)-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4k): Pale yellow solid (78% yield), Mp: 207-210 °C. FTIR (KBr, cm^{-1}): 3056 (C-H arom.), 1673 (C=O), 1518 (C=N), 1224 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.79-2.68 (m, 8H, 4CH_2 cyclohexane), 2.38 (s, 3H, $COCH_3$), 2.48 (s, 3H, NCH_3), 6.56-7.59 (m, 3H, thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.6 (CH_3), 34.8 (2CH_2), 46.8 (NCH_3), 53.2 (2CH_2), 102.9 (spiro C_2-oxad.), 134.6, 132.1, 127.9, 125.9 (4 thiophene C), 152.6 (C=N, oxad.), 167.5 (C=O). MS: *m/z* 279 [$M^{+\cdot}$]; Anal. Cald. for $C_{13}H_{17}N_3O_2S$: C, 55.89; H, 6.13; N, 15.04%. Found; C, 56.23; H 6.32; N, 14.86%.

1-Acetyl-8-isopropyl-3-(2-thienyl)-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4l): Off-white solid (73% yield), Mp: 197-199 °C. FTIR (KBr, cm^{-1}): 3058 (C-H arom.), 1675 (C=O), 1519 (C=N), 1241 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.26 (d, 6H, $2CH_3$ isopropyl), 1.81-3.00 (m, 8H, $4CH_2$ cyclohexane), 2.37 (s, 3H, $COCH_3$), 2.50 (m, 1H, NCH), 6.60-7.58 (m, 3H, thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.8 (CH_3), 27.6 ($2CH_3$), 34.4 ($2CH_2$), 47.5 (NCH), 53.1 ($2CH_2$), 101.9 (spiro C_2-oxad.), 134.8, 131.9, 128.2, 127.0, (4 thiophene C), 152.8 (C=N, oxad.), 167.3 (C=O).MS: *m/z* 307 [$M^{+\cdot}$]; Anal. Cald. for $C_{15}H_{21}N_3O_2S$: C, 58.61; H, 6.89; N, 13.67%. Found; C, 58.32; H 5.08; N, 13.86%.

1-Acetyl-8-benzyl-3-(2-thienyl)-4-oxa-1,2,8-triazaspiro[4.5]dec-2-ene (4m): White solid (66% yield), Mp: 165-167 °C. FTIR (KBr, cm^{-1}): 3060 (C-H arom.), 1674 (C=O), 1519 (C=N), 1252 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.68-2.81 (m, 8H, $4CH_2$ cyclohexane), 2.36 (s, 3H, CH_3), 3.42 (s, 2H, NCH_2), 6.58-7.95 (m, 7H, arom. + thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 22.6 (CH_3), 34.4 ($2CH_2$), 50.1 ($PhCH_2$), 53.4 ($2CH_2$), 103.8 (spiro C_2-oxad.), 140.9, 136.1, 133.6, 131.8, 128.4, 127.2, 125.8, 115.6 (8 arom. + thiophene C), 152.7 (C=N, oxad.), 167.6 (C=O). MS: *m/z* 355 [M^+]; Anal. Cald. for $C_{19}H_{21}N_3O_2S$: C, 64.20; H, 5.95; N, 11.82%. Found; C, 64.65; H 6.15; N, 11.62%.

3'-Acetyl-5'-(2-thienyl)-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4]-oxadiazole] (4n): Off-white solid (64% yield), Mp: 211-213 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1682 (C=O), 1513 (C=N), 1258 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.18-2.24 (s, 4H, $2CH_2$ indanyl), 2.41 (s, 3H, $COCH_3$), 6.59-7.98 (m, 7H, arom.+ thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 36.7 ($2CH_2$), 22.8 (CH_3), 104.8 (spiro C), 133.6, 131.2, 128.6, 127.3 (7 arom.+ thiophene C), 152.6 (C=N, oxad.), 168.2 (C=O). MS: *m/z* 298 [$M^{+\cdot}$]; Anal. Cald. for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39%. Found; C, 64.75; H 4.52; N, 9.60%.

3'-Acetyl-5'-(2-thienyl)-3,4-dihydro-2H,3'H-spiro[naphthalene-1,2'-[1,3,4]-oxadiazole] (4o): Orange solid (61% yield), Mp: 201-203 °C. FTIR (KBr, cm^{-1}): 3046 (C-H arom.), 1686 (C=O), 1519 (C=N), 1253 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 1.12-2.24 (m, 6H, $3CH_2$ tetralinyl), 2.36 (s, 3H, $COCH_3$), 6.61-8.08 (m, 7H, arom.+ thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 23.2 ($C{=}OCH_3$), 26.2, 34.0 ($2CH_2$), 49.8 (CH_2), 105.2 (spiro C), 133.6, 131.2, 128.6, 127.3 (6 arom.+ thiophene C), 152.7 (C=N, oxad.) 169.1 (C=O). MS: *m/z* 312 [$M^{+\cdot}$]; Anal. Cald. for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97%. Found; C, 65.73; H 4.96; N, 9.18%.

3'-Acetyl-5'-(2-thienyl)-3,4-dihydro-3'H,9H-spiro[9-fluorene-[1,3,4]oxadiazole] (4p): Yellow solid (64% yield), Mp: 226-229 °C. FTIR (KBr, cm^{-1}): 3076 (C-H arom.), 1682 (C=O), 1605 (C=N), 1256 (C-O-C). ^{1}H NMR (DMSO-d_6, 300 MHz; δ, ppm): 2.41 (s, 3H, $COCH_3$), 6.62-8.12 (m, 11H, arom.+ thiophene). ^{13}C NMR (DMSO-d_6, 100 MHz; δ, ppm): 24.3 (CH_3), 50.4 ($2CH_2$), 104.6 (spiro C), 133.6, 131.2, 128.6, 127.3 (10 arom. .+ thiophene C), 152.5 (C=N, oxad.), 168.7 (C=O). MS: *m/z* 346 [$M^{+\cdot}$]; Anal. Cald. for $C_{20}H_{14}N_2O_2S$: C, 69.35; H, 4.07; N, 8.09%. Found; C, 69.70; H 3.85; N, 8.31%.

III. Results and Discussion

A. Chemistry

It is worth noting that spiro-1,3,4-oxadiazole is easily produced from the nitrilimines, which are 1,3-dipoles via cycloaddition to suitable dienophile containing carbonyl group. For example, spiro[indino[1,2-b]quinoxalin-11,2′-[1,3,4]oxadiazole]-3-derivatives synthesized by one pot reaction of ninhydrin, phenylenediamine, and hydrazonoyl chloride, in the presence of Et_3N in EtOH, at room temperature [13]. 5′-aryl-3′-phenyl-3′H,12H-spiro[indolo[2,1-b]-quinazoline-6,2′-[1,3,4]oxadiazol]-12-ones were obtained from the reaction of tryptanthrins and hydrazonoyl chlorides (nitrilimines precursor) in presence of triethylamine in MeCN at 80 ºC [20]. In the presence research, the N-acylhydrazones used, were prepared by condensation of 2-furoyl hydrazine 1a or 2-thenoyl hydrazine 1b with the corresponding ketones 2a-h in refluxing ethanol producing 2-furoyl hydrazones 3a-h and 2-thenoyl hydrazones 3i-p (Scheme 1) with yields ranging 80-90%. The treatment of N-acylhydrazones 3a-p with refluxing excess acetic anhydride furnished a new series of spiro-2,3-dihydro-1,3,4-oxadiazole derivatives 4a-p (Scheme1) in very good yields, after purification by recrystallization using ethanol/ethyl acetate mixture. 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 structures of the newly synthesized compounds were confirmed through IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra studies. The synthesized compounds were found in good agreement with their spectral data. The plausible mechanism was carried out through the cycloaddition which achieved by ring formation that results from intramolecular cyclization of N-acylhydrazones 3a-p and deprotonation step forms 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-p as shown in Scheme 2. The structures of all the synthesized 1,3,4-oxadiazolines 4a-p were confirmed by elemental analysis and spectroscopic methods.

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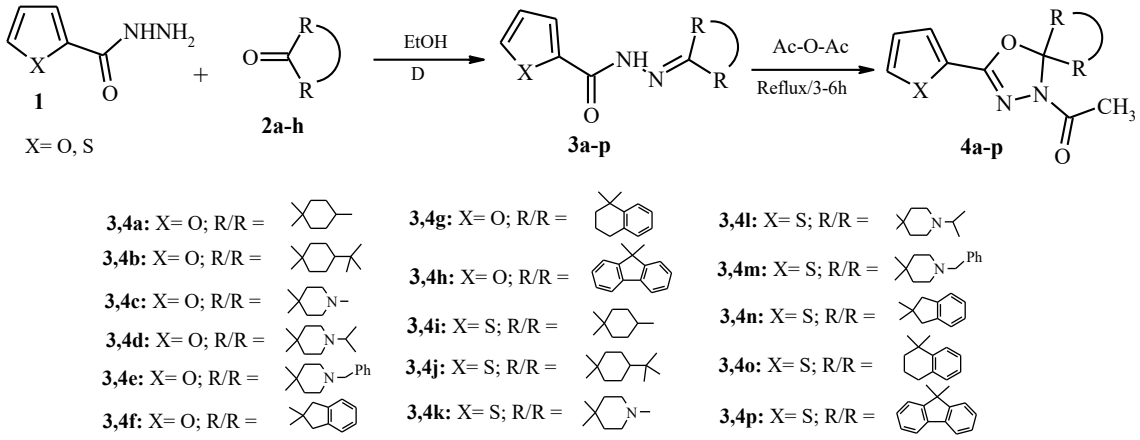

Fig. 1. Schematic synthesis of 2,3-dihydro-1,3,4-oxadiazole derivatives 4a-p.

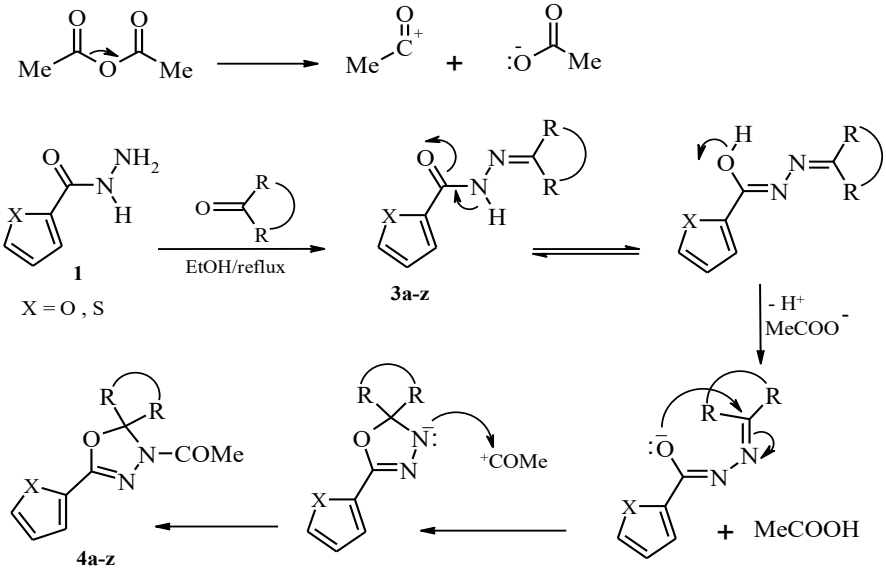

Fig. 2. A plausible mechanism for the formation of 1,3,4-oxadiazolines 4a-p

B. Spectral Data Analysis

The spectroscopic studies proved the successful acetic anhydride-promoted oxidative cyclization of N-acylhydrazones 3a-p. In the IR spectra of 1,3,4-oxadiazolines 4a-p the characteristic peak for the amide NH at 3200-3250 cm^{-1} and C=O at 1640-1650 cm^{-1} in the starting acylhydrazones 3a-p completely disappeared from the IR spectra of the obtained products 4a-p. A new C=O stretching of the acetyl group appeared at 1670-1690 cm^{-1}, C-O-C of ring at 1040-1280 cm^{-1}, and C=N stretching of dihydro-oxadiazole ring appeared in the range of 1515-1525 cm^{-1}. The second confirmation of the correct structure for compounds 4a-p, 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-p. The first evidence comes from the disappearance of the characteristic proton of the NH group at 9.0-11.0 ppm, in the ^{1}H-NMR spectra of starting hydrazones 3a-p. The disappearance of the NH proton was accompanied by the appearance of a signals at 2.3-2.6 (s), was assigned to the proton ($COCH_3$) of the acetyl group indicating the formation of the N-substituted oxadiazoline ring. In addition to the peaks for the cycloalkane ring were resonated in the aliphatic region of the spectra on the range of 2.9-1.2 ppm and aromatic protons.

The ^{13}C-NMR spectra provided an unambiguous confirmation about the formation of the 3-acetyl-2,3-dihydro-1,3,4-oxadiazole ring. In synthesized compounds 4a-p there are a new peak appeared for acetyl group carbons and this indeed are expected, because they are not part of starting hydrazones 3a-p. Where the carbonyl (C=O) of the acetyl group appeared at 166.0-169.0 ppm and the other peak appeared around 21.6-23.1 ppm is assigned to be the peaks of the CH_3 of the acetyl group. In addition to, signal at 151.2-152.2 ppm belonged to C=N carbon of cyclic oxadiazoline. Moreover, new signal at 101.0-107.0 ppm, which is attributed to spiro-carbon of oxadiazoline rings 4a-p, was of special significance in conforming the proposed structure. This is similar to the reported values of spiro-carbons flanked by heteroatoms in

oxadiazole rings [21]. 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.

C. 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 [22], [23]. 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 [23] 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 I). The activity against both bacteria and fungus were 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 I: ANTIMICROBIAL-SCREENING RESULTS OF COMPOUNDS 4A-P

Cpd. No.	Diameter of the inhibition zone in mm*						
	Antibacterial activity					Antifungal activity	
	Euterococci	*Escherichiacoli*	*Staphyloaureus*	*Klebsiella spp*	*Proteus spp*	*Candida albicans*	*Aspergillus 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
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 spiro-1,3,4-oxadiazolines 4a-s were synthesized upon the treatment of 2-furoic or 2-thienoic acid hydrazones of ketones 3a-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 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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REFERENCES

[1] Somashekhar M, Kotnal RB. Recent advances in the application of oxadiazoles in multicomponent reactions from 2015–16: A review. *Indo. Am. J. Pharm. Sci.*, 2018; 5: 7632–7644.
[2] Biernacki K, Da´sko M, Ciupak O, Rachon J, Demkowicz S. Novel 1,2,4-Oxadiazole Derivatives in Drug Discovery, *Pharmaceuticals*, 2020, 13, 111-156.
[3] Salahuddin MA, Yar MS, Mazumder R, Chakraborthy GS, Ahsan MJ, Rahman MU. Updates on synthesis and biological activities of 1,3,4-oxadiazole: A review. *Synth. Commun.*, 2017, 47, 1805–1847.
[4] Pitasse-Santos P, Sueth-Santiago V, Lima ME. 1,2,4-and 1, 3, 4-Oxadiazoles as Scaffolds in the Development of Antiparasitic Agents. *J. Braz. Chem. Soc.,* 2018; 29(3): 435-456.
[5] Glomb T, Swiatek P. Antimicrobial Activity of 1,3,4-Oxadiazole Derivatives, *Int. J. Mol. Sci.*, 2021; 22: 6979-7002.
[6] Em PC, Tuyen TN, Nguyen DH, Duy VD, Tuoi HD. Synthesis of a Series of Novel 2-Amino-5-substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole Derivatives as Potential Anticancer, Antifungal and Antibacterial Agents, *Med. Chem.*, 2022; 18(5): 558-573.
[7] Kiran S. 1,3,4-oxadiazole a potent drug candidate with various pharmacological activities, *Inter. J. Pharm. Pharm. Sc.,* 2021; 3(3): 9-16.
[8] Siwach A, Verma PK. Therapeutic potential of oxadiazole or furadiazole containing compounds. *BMC Chem.*, 2020;14(1): 70-79.
[9] Hicks C, Gulick RM. Raltegravir: The First HIV Type 1 Integrase Inhibitor. *Clin. Infect. Dise.*, 2009; 48(7): 931-939.
[10] Abu-Zaied MA, Nawwar GA, Swellem RH, El-Sayed SH. Synthesis and Screening of New 5-Substituted-1,3,4-oxadiazole-2-thioglycosides as Potent Anticancer Agents, *Pharmacol. & Pharm.*, 2012; 3: 254-261.
[11] Wang J-J, Sun W, Jia W-D, Bian M, Yu L-J. Research progress on the synthesis and pharmacology of 1,3,4-oxadiazole and1,2,4-oxadiazole derivatives: a mini review, *J. Enz. Inh. Med. Chem*., 2022; 37: 1, 2304–2319.
[12] Aiswarya G, Divekar K. A Review on Synthesis of Various Oxadiazole Derivatives Applying Green Chemistry Methods *Der Pharm. Lett*., 2021; 13(7): 108-131.
[13] Singh R, Bhardwaj D, Saini MR. Recent advancement in the synthesis of diverse spiroindeno[1,2-b]quinoxalines: a review, *RSC Adv.,* 2021; 11: 4760-4804.
[14] Alizadeh A, Moafi, L. Simple access to spirooxadiazole compounds containing a quinoxaline moiety using a nitrile imine intermediate generated in situ, *Heterocycl. Commun.,* 2017; 23: 375–378.
[15] Wang H, Pan, B, Zhang, W, Yang, C, Liu, X, Zhao, Z, et *al.* A facile and efficient synthesis of polycyclic spiropyrrolidine oxindoles bearing mesityl oxide unit via a three-component 1,3-dipolar cycloaddition reaction. *Tetrahedron*, 2015; 71: 8131–8139.
[16] Dadiboyena S, Valente EJ, Hamme AT. A novel synthesis of 1,3,5-trisubstituted pyrazoles through a spiro-pyrazoline intermediate via a tandem 1,3-dipolar cycloaddition/elimination. *Tetrahedron Lett.*, 2009; 50: 291-294.
[17] Othman AA, Kihel M, Amara S. 1,3,4-Oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole derivatives as potential antibacterial agents, *Arab. J. Chem.*, 2019; 12: 1660-1675.
[18] El-Gazzar AB, Scholten K, Guo Y, Weibenbach K, Hitzler MG, Roth G*, et al*. Cycloadditions of 1-aza-2-azoniaallene cations to isothiocyanates. *J. Chem. Soc. Perkin Trans.* 1999; 1: 1999-2010.
[19] Okimoto M, Chiba T. Electrochemical oxidation of ketone acylhydrazones and their hydrogen cyanide adducts in sodium cyanide-methanol. Transformation of ketones to nitriles, *J. Org. Chem.*, 1990; 55(3): 1070-1076.
[20] Yavari I, Askarian-Amiri M, Taheri Z. A convenient synthesis of spiro-indolo[2,1-b]quinazol-ine-6,2′-[1,3,4] oxadiazoles from tryptanthrin and nitrile imines, *Monatshefte für Chemie – Chem. Month.*, 2019; 150: 1093–1099.
[21] Morjan RY, Ghonium FJ, Abu-Teim OS, Awadallah AM, Al-Reefi MR, Elmanama A, *et al.* Syntheses of N-acylhydrazones of 2-hydroxy-3,5-dinitrobenzohydrazide, and their Conversion into 3-Acetyl-2,3-dihydro-1,3,4-oxadiazole. *IUG J.Nat. Stud.,* 2022; 30(02): 38-46.
[22] Manika M, Chanotiya C, Darokar M, Singh S, Bagchi G. Compositional characters and antimicrobial potential of Artemisia stricta Edgew. f. stricta Pamp. Essential oil. *Rec. Nat. Prod.*, 2016; 10: 40-46.
[23] *Methods for dilution antimicrobial susceptibility tests for bacteria grow aerobically. Approved Standard M7-A4*. Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 2005.