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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL 1,3,4-OXADIAZOLE BASED DITHIAZA-SPIRONES

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ABSTRACT:

A novel series of 4-phenyl-9-(5-phenyl-[1,3,4-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-diones **(4a-e)** in good to excellent yields by using commercially available 2-amino-5-phenyl-1,3,4-oxadiazole **(1)** as raw material and 2-chloro-*N*-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-acetamide **(2)** and 2-benzylidene-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-onea **(3a-e)** as intermediates. The chemical structures of these compounds have been established by IR, ^{1}H, ^{13}C-NMR, Mass spectral data and elemental analysis. The newly synthesized compounds were screened for their ability towards antimicrobial activity.

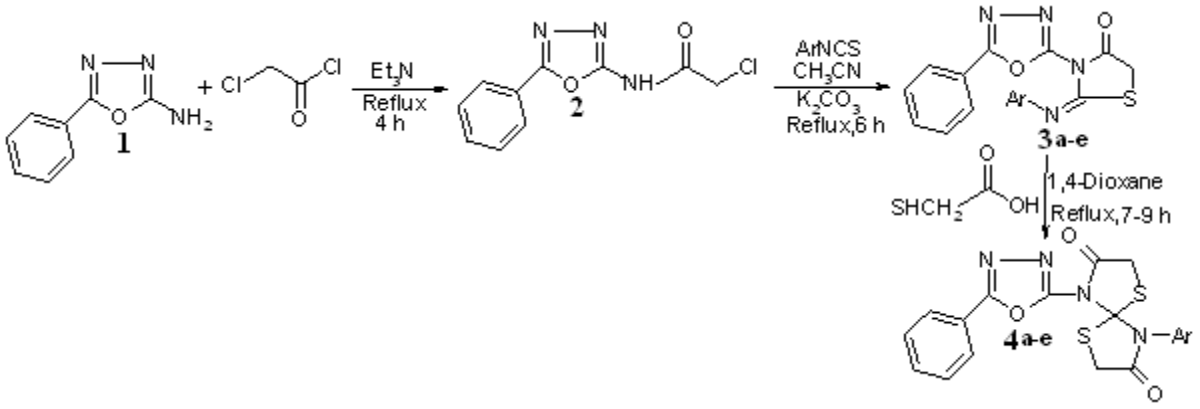
Keywords: 1,3,4-oxadiazol derivatives, Chemical structure, antimicrobial activity

INTRODUCTION

1, 3, 4-Oxadiazoles are biologically active, synthetically useful and important heterocyclic compounds. Different classes of oxadiazole compounds possess an extensive spectrum of pharmacological activities such as antibacterial[1], antimalarial[2], anti-inflammatory[3], antifungal[4], anticonvulsant[5], analgesic[6] antimycobacterial[7], antitumor[8], herbicidal[9], vasodialatory[10], cytotoxic[11], hypolipidemic[12], ulcerogenic[13] and antiedema[14]. Numerous thiazolidinone derivatives have shown significant pharmacological and biological activities[15] like sedative[16], anti-inflammatory[17], antibacterial[18], antifungal[19], antitubercular[20], anticancer[21], hypnotic[22], anti-HIV[23] and nematicidal[24].

ANTIMICROBIAL ACTIVITY

The *in vitro* antimicrobial activity of newly synthesized compounds, 4-phenyl-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-diones **(4a-e)** was determined using disc diffusion method[6]. The antibacterial activity was measured against two gram-positive strains viz., *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative strains viz., *Escherichia coli* and *Pseudomonas aeruginosa* and expressed in terms of zone of inhibition in mm at concentration of 5 µg/disc. The antifungal evaluation was carried out against fungal organisms namely *Candida albicans* and *Aspergillus niger* at concentration of 5 µg/disc. Standard antibacterial drug Ciprofloxacin (5 µg/disc) and antifungal drug Fluconazole (5 µg/disc) were also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken.



Scheme 1: 3, 4 Ar a) = C_6H_5, b) = 4-CH_3-C_6H_4, c) = 4-OCH_3-C_6H_4, d) = 4-Cl-C_6H_4, e) = 4-Br-C_6H_4

The investigation of antibacterial screening data revealed that all the tested compounds exhibited significant and interesting biological activity, however with a degree of variation. According to the results **(Table 1)**, it is clear that, some of the compounds displayed excellent antimicrobial activity. Among the series of the screened compounds **4a-e**, the compound **4e** which contain 4-bromo phenyl ring system, against *E. coli* is highly active at 28 mm zone of inhibition which is equal to the standard drug Ciproflaxacin. The compound **4e** towards *C. albicans* is also exhibited good antifungal activity at 24 mm zone of inhibition which is almost equal to the standard drug. The remaining compounds showed moderate to good activity against the test organisms. It is interesting to note that, none of the compound is inactive against all the tested microorganisms and this remarkable property may achieve to the compounds due to the two active pharmacophores (oxadiazole and thiazole) in a single molecular skeleton.

Table 1 Antimicrobial activity of compounds 4a-e (Zone of inhibition in mm)

Compound	Antibacterial activity				Antifungal activity	
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
4a	12	20	13	19	12	12
4b	11	18	10	14	13	10
4c	14	12	18	12	15	16
4d	20	21	26	20	19	20
4e	24	23	28	20	24	22
Ciprofloxacin	26	26	28	25	—	—
Fluconazole	—	—	—	—	26	25

EXPERIMENTAL

All reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a PerkinElmer BX series FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

Synthesis of 2-chloro-*N*-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-acetamide (2)

A mixture of 2-amino, 5-phenyl-1,3,4-oxadiazole **(1)** (0.01 mol) and chloroacetyl chloride (0.01 mol) was refluxed in triethyl amine (10 ml) for 4 h. After completion of the reaction (monitored by TLC), the resultant solution was cooled, the separated solid was filtered, dried and recrystallised from pet-ether to get pure 2-chloro-N-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-acetamide **(2)**.

Synthesis of 2-benzylidene-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3)

Compound **2** (0.01 mol) was treated with phenyl isocyanate (0.01 mol) at room temperature in presence of K_2CO_3 (0.5 g) in acetonitrile (15 ml). The reaction mixture was refluxed on constant stirring for 6 h. After conventional work up, the product was purified by recrystallization from methanol to give pure 2-benzylidene-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one **(3)**.

Synthesis of 4-phenyl-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4a-e)

An equimolar mixture of compound **3** (0.01 mol) and mercapto acetic acid (0.01 mol) was refluxed in dioxane for 7-9 h. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool and was poured over crushed ice. The organic layer was extracted with ethyl acetate (20 ml), washed with 10% sodium bicarbonate solution (1 X 20 ml) and dried with anhydrous sodium sulphate. The solvent was removed under vacuum and residue was recrystallized from methanol to yield the corresponding 4-phenyl-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[*4.4*]-nonane-3,8-dione **(4a-e)**.

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PHYSICAL AND SPECTRAL DATA

2-Chloro-*N*-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-acetamide (2)

White solid; Yield 74%; Mp 155-157 ^{0}C; IR (KBr) 3316 (N-H), 3024 (C-H, Ar), 2965 (C-H), 1680 (C=O), 1640 (C=N) 1560 (C=C, Ar) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (s, 2H, CH_2), 7.12-7.69 (m, 5H, Ar-H), 7.16 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 159.3, 147.2, 137.2, 134.6, 131.8 (2), 125.7 (2), 45.8; MS *m/z* 237 (M^+); Elemental analysis: Calculated for $C_{10}H_8ClN_3O_2$: C-50.54, H-3.39, Cl-14.92, N-17.68, O-13.47. Found: C-48.23, H-3.12, Cl-13.68, N-16.45, O-12.78.

2-Benzylidene-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3a)

Yellow solid; Yield 72%; Mp 163-165 ^{0}C; IR (KBr) 3035 (C-H, Ar), 1690 (C=O), 1630 (C=N), 1568 (C=C, Ar) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (s, 2H, CH_2), 6.98-7.89 (m, 10H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 161.3, 160.4, 144.8, 136.1, 134.2, 132.8, 130.6 (2), 129.3 (2), 126.8 (2), 125.3, 124.7 (2), 38.1; MS *m/z* 336 (M^+); Elemental analysis: Calculated for $C_{17}H_{12}N_4O_2S$: C-60.70, H-3.60, N-16.66, O-9.51, S-9.53. Found: C-58.79, H-3.32, N-15.64, O-8.67, S-8.74.

2-(4-Methyl-benzylidene)-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3b)

Pale yellow solid; Yield 76%; Mp 132-134 ^{0}C; IR (KBr) 3041 (C-H, Ar), 2945 (C-H, CH_3), 1685 (C=O), 1642 (C=N), 1610 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 4.26 (s, 2H, CH_2), 7.01-7.87 (m, 5H, Ar-H), 7.33 (d, 2H, *J* = 7.4 Hz, ArH), 7.45 (d, 2H, *J* = 7.4 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 160.8, 156.7, 149.2, 137.4, 135.3, 133.2, 132.7, 131.8 (2), 130.8, 129.4 (2), 126.7 (2), 124.9, 38.2, 22.6; MS *m/z* 350 (M^+); Elemental analysis: Calculated for $C_{18}H_{14}N_4O_2S$: C-61.70, H-4.03, N-15.99, O-9.13, S-9.15. Found: C-59.64, H-3.94, N-14.85, O-8.69, S-8.74.

2-(4-Methoxy-benzylidene)-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3c)

Orange solid; Yield 71%; Mp 160-162 ^{0}C; IR (KBr) 3038 (C-H, Ar), 2928 (C-H, CH_3), 1685 (C=O), 1635 (C=N), 1575 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2), 7.15-7.69 (m, 5H, Ar-H), 7.42 (d, 2H, *J* = 7.2 Hz, ArH), 7.54 (d, 2H, *J* = 7.2 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 161.8, 158.6, 155.3, 151.7, 146.4, 135.3, 134.2, 132.4, 130.7 (2), 128.6 (2), 125.8, 114.8 (2), 58.6, 23.7; MS *m/z* 366 (M^+); Elemental analysis: Calculated for $C_{18}H_{14}N_4O_3S$: C-59.01, H-3.85, N-15.29, O-13.10, S-8.75. Found: C-57.62, H-3.27, N-14.39, O-12.86, S-7.95.

2-(4-Chloro-benzylidene)-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3d)

Brown solid; Yield 73%; Mp 150-152 ^{0}C; IR (KBr) 3028 (C-H, Ar), 1695 (C=O), 1630 (C=N), 1584 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (s, 2H, CH_2), 7.09-7.54 (m, 5H, Ar-H), 7.39 (d, 2H, *J* = 7.0 Hz, ArH), 7.56 (d, 2H, *J* = 7.0 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 160.8, 158.7, 151.0, 143.7, 133.8, 131.2, 130.7, 129.4 (2), 128.7, 127.6 (2), 125.4 (2), 123.6, 35.7; MS *m/z* 370 (M^+); Elemental analysis: Calculated for $C_{17}H_{11}ClN_4O_2S$: C-55.06, H-2.99, Cl-9.56, N-15.11, O-8.63, S-8.65. Found: C-53.84, H-2.27, Cl-8.72, N-14.39, O-7.86, S-7.84.

2-(4-Bromo-benzylidene)-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one (3e)

Pale yellow solid; Yield 74%; Mp 144-146 ^{0}C; IR (KBr) 3032 (C-H, Ar), 1680 (C=O), 1631 (C=N), 1578 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (s, 2H, CH_2), 7.12-7.68 (m, 5H, Ar-H), 7.42 (d, 2H, *J* = 7.4 Hz, ArH), 7.74 (d, 2H, *J* = 7.4 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 161.6, 159.6, 156.7, 149.2, 135.3, 1342, 133.7, 132.8 (2), 131.7, 130.5 (2), 126.7 (2), 124.9, 38.2, MS *m/z* 415 (M^+); Elemental analysis: Calculated for $C_{17}H_{11}BrN_4O_2S$: C-49.17, H-2.67, Br-19.24, N-13.49, O-7.71, S-7.72. Found: C-47.36, H-2.32, Br-17.46, N-12.39, O-6.94, S-6.84.

4-Phenyl-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4a)

Brown solid; Yield 70%; Mp 170-172 ^{0}C; IR (KBr) 3048 (C-H, Ar), 1710 (C=O), 1634 (C=N), 1610 (C=C, Ar), 1275 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.18 (s, 2H, CH_2), 4.41 (s, 2H, CH_2), 7.12-7.69 (m, 10H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 164.8, 153.2, 144.6, 141.6, 137.4, 131.8 (2), 129.7, 127.6 (2), 126.3 (2), 123.4, 121.7 (2), 76.4, 32.0, 28.6; MS *m/z* 410 (M^+); Elemental analysis: Calculated for $C_{19}H_{14}N_4O_3S_2$: C-55.60, H-3.44, N-13.65, O-11.69, S-15.62. Found: C-54.36, H-3.14, N-12.95, O-10.74, S-14.82.

4-(5-Phenyl-[1,3,4]-oxadiazol-2-yl)-9-p-tolyl-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4b)

Yellow solid; Yield 77%; Mp 140-142 ^{0}C; IR (KBr) 3036 (C-H, Ar), 1710 (C=O), 1634 (C=N), 1623 (C=C), 1285 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 4.21 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 7.12-7.59 (m, 5H, Ar-H), 7.42 (d, 2H, *J* = 7.4 Hz, ArH), 7.68 (d, 2H, *J* = 7.4 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 162.7, 155.4, 146.7, 143.0, 139.4, 134.2, 132.6 (2), 130.2, 127.6 (2), 125.8 (2), 120.9 (2), 74.8, 30.7, 26.4, 22.7; MS *m/z* 424 (M^+); Elemental analysis: Calculated for $C_{20}H_{16}N_4O_3S_2$: C-56.59, H-3.80, N-13.20, O-11.31, S-15.11. Found: C-54.95, H-3.25, N-12.94, O-10.85, S-14.67.

4-(4-Methoxy-phenyl)-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4c)

Orange solid; Yield 71%; Mp 120-122 ^{0}C; IR (KBr) 3038 (C-H, Ar), 1720 (C=O),1642 (C=N), 1624 (C=C), 1280 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (s, 3H, OCH_3), 4.06 (s, 2H, CH_2), 4.19 (s, 2H, CH_2), 7.21-7.78 (m, 5H, Ar-

H), 7.51 (d, 2H, *J* = 7.0 Hz, ArH), 7.65 (d, 2H, *J* = 7.0 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 163.2, 154.8, 146.7, 143.7, 139.6, 136.7, 134.5 (2), 132.7 (2), 130.2, 128.7 (2), 123.4 (2), 78.7, 30.3, 26.3, 21.7; MS *m/z* 440 (M^+); Elemental analysis: Calculated for $C_{20}H_{16}N_4O_4S_2$: C-54.53, H-3.66, N-12.72, O-14.53, S-14.56. Found: C-52.68, H-3.21, N-11.95, O-13.64, S-13.74.

4-(4-Chloro-phenyl)-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4d)

Yellow solid; Yield 70%; Mp 132-134 ^{0}C; IR (KBr) 3051 (C-H, Ar), 1648 (C=N), 1715 (C=O), 1623 (C=C),1270 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.18 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 7.12-7.59 (m, 5H, Ar-H), 7.42 (d, 2H, *J* = 7.8 Hz, ArH), 7.65 (d, 2H, *J* = 7.8 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 163.2, 151.7, 142.8, 138.7, 135.4, 131.7 (2), 129.6, 128.7 (2), 127.2, 125.3 (2), 119.8 (2), 73.7, 35.4, 29.7; MS *m/z* 444 (M^+); Elemental analysis: Calculated for $C_{19}H_{13}ClN_4O_3S_2$: C-51.29, H-2.95, Cl-7.97, N-12.59, O-10.79, S-14.41. Found: C-49.36, H-2.24, Cl-6.98, N-11.94, O-9.94, S-13.75.

4-(4-Bromo-phenyl)-9-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione (4e)

Orange solid; Yield 73%; Mp 114-116 ^{0}C; IR (KBr) 3048 (C-H, Ar), 1720 (C=O),1660 (C=N), 1625 (C=C), 1275 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (s, 2H, CH_2), 4.26 (s, 2H, CH_2), 7.18-7.58 (m, 5H, Ar-H), 7.39 (d, 2H, *J* = 7.8 Hz, ArH), 7.68 (d, 2H, *J* = 7.8 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 160.7, 151.8, 140.6, 138.9, 135.7, 132.8 (2), 129.8 (2), 126.8, 123.7 (2), 120.1 (2), 119.3, 78.7, 36.3, 30.2; MS *m/z* 487 (M^+); Elemental analysis: Calculated for $C_{19}H_{13}BrN_4O_3S_2$: C-46.63, H-2.68, Br-16.33, N-11.45, O-9.81, S-13.10. Found: C-44.39, H-2.31, Br-15.64, N-10.75, O-9.28, S-12.46.

RESULTS AND DISCUSSION

The therapeutic importance of these rings prompted us to develop selective molecules in which a substituent could be arranged in a pharmacophoric pattern to display higher pharmacological activities. Thus we have designed and synthesized a series of novel 4-phenyl-9-(5-phenyl-[1,3,4-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-dione **(4)** in good to excellent yields by using commercially available 2-amino-5-phenyl-1,3,4-oxadiazole **(1)**. The synthetic route leading to the title compounds is summarized in scheme 1.

The initial intermediate, 2-chloro-*N*-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-acetamide **(2)** was prepared through dehydrohalogenation between compound **1** and chloroacetyl chloride in triethyl amine under reflux for 4 h. Compound **2** on cyclization with various phenyl thioisocyanates in presence of potassium carbonate in refluxing acetonitrile for 6 h to get the corresponding intermediate, 2-benzylidene-3-(5-phenyl-[1,3,5]-oxadiazol-2-yl)-thiazolidin-4-one **(3a-e)**. The title compounds 4-phenyl-9-(5-phenyl-[1,3,4-oxadiazol-2-yl)-1,6-dithia-4,9-diaza-spiro-[4.4]-nonane-3,8-diones **(4a-e)** have been prepared from the subsequent ring closure reaction of compound **3** with mercapto acetic acid in refluxing 1,4-diaxane for 7-9 h. The chemical structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H, ^{13}C NMR, mass spectral data and elemental analysis. Further the compounds **4a-e** were used to evaluate their antimicrobial activity.

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