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Research Article

Design and Therapeutic Potential of 2,5-substituted diphenyl-1,3,4-oxadiazole Derivatives

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ABSTRACT

In the present study, new class of 2,5-substituted diphenyl-1,3,4-oxadiazole derivatives were developed by combining substituted aromatic acids with hydrazine hydrate in the presence of $POCl_3$ reagent under various reaction conditions. The compounds were also evaluated for their antimicrobial potential against some bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Aspergillus niger*). As the result of the newly synthesized compounds were characterized as 2-(4-chlorophenyl) compounds-5-(4-nitrophenyl)-1,3,4-oxadiazole (13), 2-(4-methylphenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (16), 2-(4-chlorophenyl)-5-(4-methoxyhenyl)-1,3,4-oxadiazole (17), 4-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]phenol (18), and 2-(4-chlorophenyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (25). The results also revealed that these compounds have good potential to inhibit microbes. It may be concluded that oxadiazole nucleus may be a profitable source for the synthesis of drugs against infectious diseases.

Introduction

Some medicinally significant derivatives containing pyrazoles such as novalgina, aminopyran, and other commonly used medications similar to nonsteroidal anti-inflammatory drugs (NSAIDs).[1] Antifungal, antimalarial, and hypertensive are only a few of the biological effects that imidazoles and triazoles, respectively, can cause.[2] Recently, it has become the main attention of medicinal chemists that the presence of oxygen and nitrogen in heterocyclic systems is particularly interesting. This is due to the extensive range of biological processes involved in oxygen and nitrogen and the remarkable effectiveness of these systems. In keeping with this notion, it became clear that oxadiazoles have gained a lot of interest due to their very powerful antibacterial qualities. This came about as a result of the fact that they contain these capabilities.[3-4]

It has been demonstrated that the oxadiazole nucleus is a profitable target for the creation of drugs due to the high amount of bioactivity it possesses. Consequently, the production of 1,3,4-oxadiazole and its transformation have continuously been the subject of a significant amount of research or a significant amount of time. It has been used to identify compounds that have the 1,3,4-oxadiazole ring, a key component of pharmaceutical chemistry with important biological effects, antibacterial,[5-8] antituberculosis,[9-11] anticancer,[12] anti-inflammatory,[13-14] antiparasitic,[15] and antihyperglycemic drug.[16] This ring was discovered through the use of this technique, that is, cyclization of substituted benzo hydrazide to 1,3,4-oxadiazole. It has also been used to identify compounds with antiparasitic and antibacterial properties. In addition, it has also been shown that it can cause cell apoptosis.[17]

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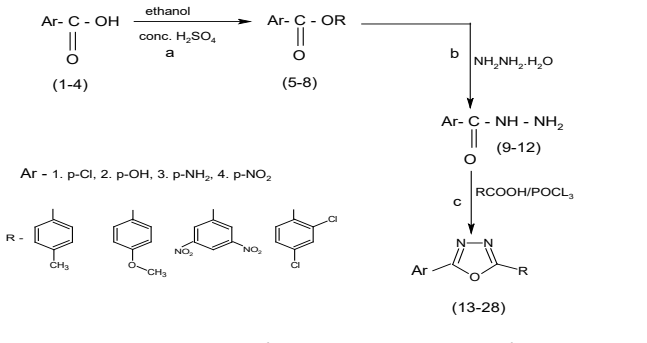
Antimicrobial resistance is responsible for the death of at least 1.27 million individuals in 2019, and it is anticipated that the actual figure will be closer to 5 million. Because of this, the well-being of every person on the planet has placed in a difficult position.[18] There is a chance that antimicrobial resistance will affect people in all phases of their lives and in the medical, veterinary, and agricultural industries. In light of this, it is one of the most serious threats to the general population's health that exists everywhere in the world. Antimicrobial resistance occurs when microbes like bacteria, fungus, and other organisms develop the ability to withstand the effects of antimicrobial drugs.[19] This creates the impression that the germs are thriving even after being killed. Infectious agents are acquiring a higher level of resistance to therapies that are now available on the market as a direct result of the increased usage of antibiotics over the past several years. Even if there has been an increase in the number of illnesses that are immune to the therapies that are now available worldwide, there is still a need for new antimicrobial drugs. In addition, germs that have developed the ability to resist the effects of many medications pose a considerable risk to the well-being of human beings. The usage of genomes may lead to the discovery of new therapeutic targets, the improvement of antibiotics that are already on the market, and, most critically, the discovery of new antimicrobial treatments.[20-22]

So, in todays scenario, there is a need to find more compounds to control infectious diseases as mostly drugs microbes have developed resistance, which are currently being used. These compounds should be produced in bulk quantity in lesser time without side effects. So, in the present investigation, the synthesis of substituted 1,3,4-oxadiazole derivatives were carried out which were further evaluated for their antibacterial and antifungal potential.

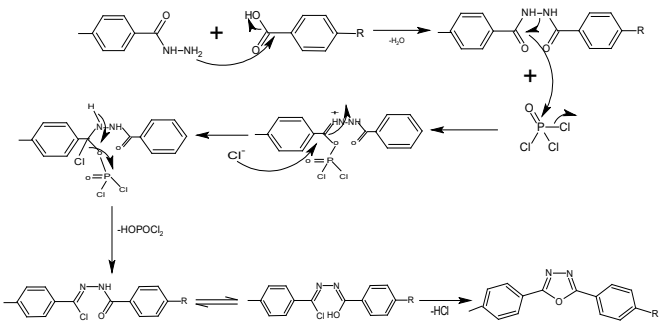
Material And Methods

The compounds were purchased commercially and used without further purification. The reaction was monitored using a technique called thin layer chromatography. This technique was carried out on plates Merck had precoated with a silica gel compound called G. The product contains ethyl acetate and petroleum ether in its composition. As solvent systems, we employed benzene and methanol (4:6 volume/volume) and benzene and methanol (3:2 volume/volume). To make the dots visible, ultraviolet light and iodine vapors were utilized. The melting point was calculated using a method involving an open capillary tube, and the findings were not subjected to any adjustment in any way. A Thermos Nicolet Avatar 330-FTIR spectrophotometer was used to get the IR spectra using KBr. The ^{1}H-NMR spectra were acquired using a Bruker Advance Neo 500MHz NMR spectrophotometer, $CDCl_3$ as the solvent, and tetramethyl silane (TMS) as the internal standard.



Reaction conditions : (a) reflux 6 h at 40-50^{0}C (b) stirring/15min, reflux 9 h at 30-40^{0}C; (c) reflux 3 h at 100^{0}C, crushed ice

Scheme 1: Synthetic scheme of the 2,5-substituted-1,3,4-oxadiazole derivatives



Scheme 2: Proposed mechanism for the cyclization of substituted benzo hydrazide to 1,3,4-oxadiazole

This was done to produce accurate results. The delta scale is utilized to display the chemical shift values. Analyzers from the FLASH EA 1112 series CHNS-O were used for the elemental analysis, and an Agilent 1100 series LC-MS was used to record the mass spectra. Both of these instruments were from FLASH. The mechanisms that result in the formation of the required heterocyclic molecules are depicted in Scheme 1, which is available to the reader and cyclization mechanism is represented in the Scheme 2.

General Steps for Synthesizing 2,5-disubstituted derivatives of 1,3,4-oxadiazole

STEP-1: Substituted Ethyl Benzoate Synthesis (5-8)

Concisely and drop by drop suitable substituted benzoic acid (0.0623 mol) (1-4) that had been dissolved in ethanol (12 mL) was used to form the reaction mixture. Additionally, 0.5 mL of sulphury acid was added to the mixture. After heating to an internal temperature of between 40–50°C, the mixture was allowed to reflux for six hours. After an excessive amount of alcohol was distilled and cooled by submerged in ice water. We made use of TLC so that we could monitor how far along the reaction was progressing as it was taking place. After the cooling process, the combination was finished; it was filtered, washed with water, and recrystallized with ethanol. The information that can be found in Table 1 pertains to the physiochemical characteristics of the compounds that were synthesized in a laboratory (5-8).

Table 1: Physiochemical data of the synthesized compound (5-8)

Compound	*Ar*	*Molecular formula*	*Mw.*	*% Yield*	*m.p. (°C)*	*Rf solvent system*
5	p-Cl	$C_9H_9ClO_2$	184.62	70.23	126–128	0.75[a]
6	p-OH	$C_9H_{10}O_3$	166.17	40.35	110–112	0.69[a]
7	p-NH_2	$C_9H_{11}NO_2$	165.19	58.89	92–94	0.85[a]
8	p-NO_2	$C_9H_9NO_4$	195.17	90.24	65–67	0.79[a]

[a] Ethyl acetate: petroleum ether (4:6 v/v)

STEP 2: Substituted Benzo Hydrazide Synthesis (9-12)

Compounds 9–12 were produced as a result of a reaction in which substituted ethyl benzoate (0.0723 mol) (5-8) and hydrazine hydrate (6 mL) were refluxed together for 9 hours at temperatures ranging from 30–40°C. The liquid was allowed to reach at room temperature before being poured onto the crushed ice. After allowing the temperature to return to normal, this was carried out. TLC was utilized to keep track of the reaction being observed and maintain track of the reaction being monitored. After filtering and washing the residue with water, ethanol was employed to recrystallize it so that the final product would have crystals. The results of this technique were a material that had a crystalline structure. Table 2 presents the physiochemical data for the newly synthesized compounds (9–12).

STEP 3: 2,5-Substituted-1,3,4-Oxadiazole Derivatives Synthesis (13-28)

Phosphorous oxy chloride (6 mL) at temperature of 100°C with an equimolar mixture consisting of substituted benzo hydrazide, a combination of aside (9–12) (0.0064 mol) with several various substituted benzoic acids (0.0064 mol) was refluxed. Before carrying on with the experiment, the reaction mixture temperature was decreased to room temperature. Then, on top of the ice, it was poured. To produce derivatives of the 1,3,4-oxadiazole ring with a 2,5-substituted position, The precipitate was removed, washed with water, and then recrystallized with ethanol before being utilized to make derivatives. The physiochemical characteristics of the newly synthesized compounds are summarized in Table 3, which may be found below (13-28).

Characterization Data of Compounds (13-28)

2-(4-chlorophenyl)-5-(4-methylphenyl) -1,3,4-oxadiazole (13)

^{1}H-NMR (400MHz-$CDCl_3$): δ 2.29 (3H, s, CH_3), 7.44 (2H, Ar-H did, J = 7.7, 1.4, 0.4 Hz), 7.85-8.05 (6H, Ar-H 7.91 (did, J = 8.2, 1.5, 0.4 Hz). IR (KBr, cm^{-1}): 3042.0 (Ar-H str.), 3182.4 (C-H aliphatic str.), 1651.6 (C=C str.), 1457.4 (C=N str.), 1348.9 (C-O-C ox. Str.), 1245.3 (N-N oxa. Str.), 1063. 6 (=C-O str.), 852.0 (C-Cl str.). MS (EI) m/z 270.05 [M+], Analytical Calculations for $C_{15}H_{11}ClN_2O$ found: C-65.43, H-3.94, N-10.01, Cl-13.00, and O-5.44 %.

4-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl] phenol (14)

^{1}H-NMR (400 MHz-$CDCl_3$): δ 2.34 (3 H, s, CH_3) 7.24–7.47 (4 H, Ar–H 7. 30 (did, J = 8.6, 1.3, 0.4 Hz), 7.41 (did, J = 7.7, 1.3, 0.4 Hz)), 7.90-8.05 (4H, 7.97 (did, J = 7.7, 1.8, 0.4 Hz). IR (KBr, cm^{-1}): 3616.6 (O-H str.), 3189.2 (Ar-H str.), 3042.0 (C-H aliphatic str.), 1558.8 (C=C str.), 1457.5 (C=N str.), 1349.0 (C-O-C ox. Str.), 1244.5 (N-N ox), 1065.2 (=C-O str. MS (EI) m/z 252.09 [M+], Analytical Calculated For: $C_{15}H_{12}N_2O_2$; C- 71.42, H- 4.79, N-11.1, O-12.68, Found: C- 69.43, H- 4.02, N-10.30, O-11.44 %.

4-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl] aniline (15)

^{1}H-NMR (400MHz-$CDCl_3$): δ 2.35 (3H, s, CH_3), 6.73 (2H, Ar-H did, J = 8.5, 1.3, 0.4 Hz), 7.38 (2H, did, J = 7.8, 1.2, 0.4 Hz), 7.76 (2H, did, J = 8.5, 1.6, 0.4 Hz), 7.89 (2H, did, J = 7.8, 1.8, 0.4 Hz). IR (KBr, cm^{-1}): 3457.9 (N-H str.), 3186.8 (Ar-H str.), 3040.7 (C-H aliphatic str.), 1568.3 (C=C str.), 1482.2 (C=N str.), 1348.6 (C-O-C ox. str.), and 1243.4 (N-N ox), 1065.5 (=C-O str.). MS (EI) m/z 251.10 [M+], Analytical Calculated For: $C_{15}H_{13}N_2O$; C-71.7, H-5.21, N-16.72, O-6.37, Found: C-70.21, H-4.94, N-15.01, O-5.46 %

2-(4-methylphenyl)-5-(4-nitrophenyl) -1,3,4-oxadiazole (16)

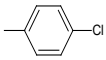
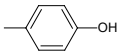
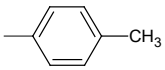
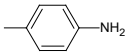
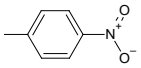
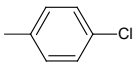
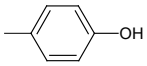
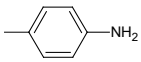
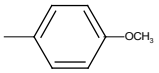
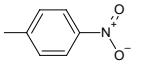
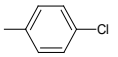
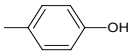
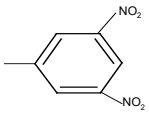
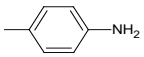
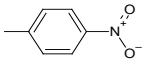
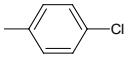
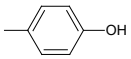
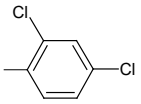
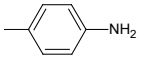
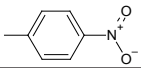
^{1}H-NMR (400MHz-$CDCl_3$): δ 2.34 (3H, s, CH_3), 7.13 (2H, Ar-H did, J = 8.7, 1.4, 0.4 Hz), 7.40 (2H, did, J = 7.7, 1.3, 0.4 Hz),7.90-8.06 (4H, 7.96 (did, J = 7.7, 1.8, 0.4 Hz), IR (KBr, cm^{-1}): 3186.6(Ar-H str), 3038.0(C-H aliphatic str.), 1567.7(C=C str), 1485.8(C=N str.), 1567.7(-NO_2 str.), 1350.1(C-O-C ox. Str.), 1250.2(N-N ox), 1058.8(=C-O str.), MS (EI) m/z 283.09 [M+], nalytical Calculated For: $C_{15}H_{11}N_3O_3$; C-64.05, H- 3.94, N-14.94, O-17.06, Found: C-64.23, H-3.12, N-15.11, O-16.21 %.

Table 2: Physiochemical data of the synthesized compound (9-12)

Compound	*Ar*	*Molecular Formula*	*Mw*	*% Yield*	*m.p. (°C)*	*Rf solvent system*
9	p-Cl	$C_7H_7ClN_2O_2$	170.60	55.24	161–163	0.78[a]
10	p-OH	$C_7H_8N_2O_2$	152.1	38.20	136–138	0.72[a]
11	p-NH_2	$C_7H_9N_3O$	151.17	50.69	210–212	0.80[a]
12	p-NO_2	$C_7H_7N_3O_3$	181.15	85.37	150–152	0.83[a]

[a]Ethyl acetate: Petroleum ether (4:6 v/v)

Table 3: Physio-chemical data of the synthesized compounds (13-28)

Compound	*Ar*	*R*	*M wt*	*%Yield*	*m. p. (°C)*	*Rf solvent system*
13			270.71	73.11	101–103	0.78a
14			252.26	56.90	131–133	0.67a
15			251.28	62.45	154–156	0.88a
16			281.3	83.90	165–167	0.97a
17			286.71	78.23	103–105	0.65a
18			268.27	60.34	95–97	0.76a
19			267.28	55.44	110–113	0.88a
20			297.27	92.32	134–136	0.86a
21			490.9	75.98	123–125	0.61a
22			328.23	64.34	178–179	0.55b
23			327.25	68.88	106–108	0.79a
24			357.24	86.66	110–113	0.51a
25			325.6	80.24	127–129	0.67a
26			307.1	69.87	97–99	0.82b
27			306.1	70.34	102–104	0.86b
28			336.1	95.23	120–122	0.70b

[a]ethyl benzoate: Petroleum ether (4:6 v/v)
[b]enzene: Methanol (3:2 v/v)

2-(4-chlorophenyl)-5-(4-methoxyphenyl) -1,3,4-oxadiazole (17)

^{1}H-NMR (400MHz-$CDCl_3$): δ 3.85 (3H, s, CH_3),7.31 (2H, Ar-H did, J = 8.6, 1.3, 0.4 Hz), 7.81-8.04 (6H, 7.88 (did, J = 8.2, 1.3, 0.4 Hz), IR (KBr, cm^{-1}): 3191.7(Ar-H str), 3010.8(C-H aliphatic str.), 1603.7(C=C str), 1457.0(C=N str.), 1347.2(C-O-C ox. Str.), 1243.0(N-N oxa), 1064.6(=C-O str.), 1044.6(C-Cl str.), MS (EI) m/z 286.05 [M+], Anal. Calcd. For: $C_{15}H_{11}ClN_2O_2$; C- 62.84, H- 3.87, N-9.77, Cl-12.36, O-11.16, Found: C-61.33, H-3.94, N-10.01, Cl-13.00, O-10.21%.

4-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl] phenol (18)

NMR ^{1}H (400MHz-$CDCl_3$): δ 3.83 (3H, s, CH_3), 7.17-7.32 (4H, Ar-H 7.23), (did, J = 8.6, 1.2, 0.4 Hz), 7.25 (did, J = 8.6, 1.2, 0.4 Hz)), 7.69-7.82 (4H, 7.75 (did, J = 8.6, 1.6, 0.4 Hz), IR (KBr, cm^{-1}): 3618.0(O-H str.), 3191.4(Ar-H str), 3010.3(C-H aliphatic str.), 1655.3(C=C str), 1457.4(C=N str.), 1348.5(C-O-C ox. Str.), 1242.3(N-N ox), 1066.0(=C-O str.), 912.3(O-CH_3 str.), MS (EI) m/z 268.08 [M+], Analytical calculations for $C_{15}H_{12}N_2O_3$ C-67.16, H-4.51, N-10.44, and O-17.89, found: C-66.09, H-4.94, N-10.90, and O-15.44%.

4-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl] aniline (19)

^{1}H-NMR (400MHz-$CDCl_3$): δ 3.82 (3H, s, CH_3), 6.73 (2H, Ar-H did, J = 8.5, 1.2, 0.4 Hz), 7.25 (2H, did, J = 8.8, 1.2, 0.4 Hz), 7.65 (2H, did, J = 8.5, 1.6, 0.4 Hz), 7.83 (2H, did, J = 8.8, 1.6, 0.4 Hz). IR (KBr, cm^{-1}): 3459.6 (N-H str), 3191.2 (Ar-H str), 3011.2 (C-H aliphatic str.), 1535.2 (C=C str), 1455.8 (C=N str.), 1347.5 (C-O-C ox. Str.), 1241.2 (N-N oxa), 1065.7 (=C-O str.), 912.2 (O-CH3 str), MS (EI) m/z 267.10 [M+], Anal. Clad. For: $C_{15}H_{13}N_3O_2$; C-67.40, H-4.9, N-15.72, and O-11.97, Found: C-67.00, H-4.09, and N-14.90 O-11.82%.

2-(4-methoxyphenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (20)

^{1}H-NMR (400MHz-$CDCl_3$): δ 3.85 (3H, s, CH_3) and 7.12–7.26 (4H, Ar–H 7.19). (Did, J = 8.6, 1.5, 0.4 Hz), 7.20 (did, J = 8.6,

1.2, 0.4 Hz)), 7.68-7.87 (4H, 7.75 (did, J = 8.6, 1.6, 0.4 Hz). IR (KBr, cm^{-1}): 3191.4(Ar-H str), 3008.4(C-H aliphatic str.), 1602.5(C=C str), 1483.6(C=N str.), 1519.7(-NO2 str.), 1351.3(C-O-C ox. Str.), 1245.4(N-N ox), 1062.6(=C-O str.), MS (EI) m/z 299.09 [M+], Analytical calculations $C_{15}H_{11}N_3O_4$; C- 60.61, H- 3.73, N-14.14, O-21.53, Found: C-59.99, H-3.94, N-15.01, O-20.33%.

2-(4-chlorophenyl)-5-(3,5-dinitrophenyl)-1,3,4-oxadiazole (21)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.27 (1H, Ar-H t, J = 2.7 Hz), 7.72-7.94 (6H, Ar-H 7.78 (dd, J = 2.7, 1.9 Hz), 7.87 (did, J = 7.9, 1.6, 0.4 Hz). IR (KBr, cm^{-1}): 3627.0(Ar-H str), 1644.0(C=C str), 1538.6(C=N str.), 1342.0(C-O-C ox. Str.), 1245.7(N-N oxa), 1059.2(=C-O str.), 1044.4(C-Cl str.), 1359.0 (N-O str). MS (EI) m/z 346.02 [M+]; Analytical Calculated For: $C_{14}H_7ClN_3O_5$; C- 50.55, H- 2.12, N-12.63, Cl-10.66, O-24.05; Found: C-50.00, H- 2.01, N-12.90, Cl-10.00, O-23.44%.

4-[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl] phenol (22)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.07 (2H, Ar-H did, J= 8.4, 1.1, 0.4 Hz), 7.24 (1H, Ar-H t, J= 2.6 Hz), 7.64-7.83 (4H, 7.70 (dd, J = 2.6, 1.9 Hz). IR (KBr, cm^{-1}): 3624.0(O-H str.), 3193.3(Ar-H str), 1556.3(C=C str), 1540.2(C=N str.), 1377.8(C-O-C ox. Str.), 1246.5 (N-N ox), 1059.6(=C-O str.), 1359.0(-NO2 str.), MS (EI) m/z 328.044 [M+], Analytical Calculated For: $C_{14}H_8N_4O_6$; C- 51.23, H-2.46, N-17.07, O-29.25, Found: C-50.43, H-2.94, N-16.09, O-28.84%

4-[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl] aniline (23)

^{1}H-NMR (400MHz-$CDCl_3$): δ 6.73 (2H, Ar-H did, J = 8.2, 1.2, 0.4 Hz), 7.16 (1H, Ar-H t, J = 2.7 Hz), 7.60-7.79 (4H, 7.67 (did, J = 8.2, 1.6, 0.4 Hz).IR (KBr, cm^{-1}): 3453.4(N-H str.), 3195.6(Ar-H str), 1631.2(C=C str), 1465.7(C=N str.), 1374.8 (C-O-C ox. Str.), 1246.8(N-N oxa), 1060.3(=C-O str.), 1459.0(-NO2 str.), Anal. MS (EI) m/z 327.07 [M+], Anal. Calcd. For: $C_{14}H_9N_5O_5$; C- 51.38, H- 2.77, N-21.4, O-24.44, Found: C-50.43, H-2.14, N-20.01, O-23.32%.

2-(3,5-dinitrophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (24)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.19-7.39 (3H, Ar-H 7.25 (t, J = 2.6 Hz), 7.32 (ddd, J = 8.4, 1.4, 0.4 Hz)), 7.70 (2H, Ar-H dd), 7.82 (2H, Ar-H ddd). IR (KBr, cm^{-1}): 3193.1 (Ar-H str), 1505.3(C=C str), 1468. 2(C=N str.), 1603.0 (-NO2 str.),1350.4(C-O-C oxa. Str.), 1247.8(N-N oxa), 1060.1(=C-O str.), MS (EI) m/z 357.03 [M+], Anal. Calcd. For: $C_{14}H_7N_5O_7$; C- 47.07, H- 1.98, N-19.60, O-31.35, Found: C-45.90, H-1.94, N-18.41, O-32.44%.

2-(4-chlorophenyl)-5-(2,4-chlorophenyl)-1,3,4-oxadiazole (25)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.31 (1H, Ar-H dd, J = 1.8, 0.4 Hz), 7.60 (1H, Ar-H dd, J = 8.2, 1.8 Hz), 7.87-8.05 (5H, Ar-H 7.93 (ddd, J = 8.2, 1.6, 0.4 Hz) IR (KBr, cm^{-1}) values: 3184.4 (Ar-H str), 1646.4 (C=C str), 1548.3 (C=N str.), 1337.9 (C-O-C oxa. Str.), 1241.3 (N-N ox), 1065.4 (=C-O str.), 1044.9 (C-Cl str.), MS (EI) m/z 323.96 [M+], Analytical calculations for $C_{14}H_7Cl_3N_2O$; C - 51.65, H - 2.17, N -8.6, Cl -32.67, and O -4.91. Found: C -50.98, H -1.94, N -8.01, Cl -33.00, and O 4.44%.

4-[5-(2,4-chlorophenyl)-1,3,4-oxadiazol-2-yl] phenol (26)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.24-7.40 (3H, Ar-H 7.31 (did, J = 8.6, 1.2, 0.4 Hz),7.34 (dd, J = 1.8, 0.4 Hz)), 7.58 (1H, dd, J = 8.2, 1.8 Hz), 7.90-8.04 (3H, Ar-H 7.96 (dd, J = 8.2, 0.4 Hz). IR (KBr, cm^{-1}): 3620.9(O-H str.), 3187.8(Ar-H str), 1584.8(C=C str), 146.3(C=N str.), 1438.2 (C-O-C ox. Str.), 1240.7(N-N ox), 1066.1(=C-O str.), 1064.6(C-Cl str.), MS (EI) m/z 305.99 [M+], Anal. Clad. For: $C_{14}H_8Cl_2N_2O_2$; C- 54.75, H- 2.63, N-9.12, Cl-23.08, O-10.42, Found: C-53.53, H-2.14, N-10.01, Cl-23.00, O-10.14%.

4-[5-(2,4-chlorophenyl)-1,3,4-oxadiazol-2-yl] aniline (27)

^{1}H-NMR (400MHz-$CDCl_3$): δ 6.74 (2H, did, J = 8.5, 1.2, 0.4 Hz), 7.33 (1H, dd, J = 1.7, 0.4 Hz), 7.60-7.76 (3H, 7.67 (dd, J = 8.2, 1.7 Hz), 7.87 (1H, dd, J = 8.2, 0.4 Hz). IR (KBr, cm-1): 3510.6(N-H str.), 3188.0(Ar-H str), 1633.8(C=C str), 1544.8(C=N str.), 1339.2 (C-O-C ox. Str.), 1239.9(N-N oxa), 1066.0(=C-O str.), 1072.8 (C-Cl str.), MS (EI) m/z 305.01 [M+], Anal. Calcd. For: $C_{14}H_9Cl_2N_3O$; C- 54.93, H- 2.96, N-13.73, Cl-23.16, O-5.23, Found: C-53.90, H-2.94, N-12.98, Cl-23.90, O-4.44%.

2-(2,4-chlorophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (28)

^{1}H-NMR (400MHz-$CDCl_3$): δ 7.22-7.40 (3H, Ar-H 7.28 (did, J = 8.7, 1.7, 0.4 Hz), 7.34 (dd, J = 1.8, 0.4 Hz)), 7.58 (1H, Ar-H dd, J = 8.2, 1.8 Hz), 7.86-8.02 (3H, Ar-H 7.93 (did, J = 8.7, 1.7, 0.4 Hz). IR (KBr, cm^{-1}): 3183.9 (Ar–H str), 1585.0 (C–C str), 1467.0 (C–N str.), 1601.1 (-NO_2 str.), ,1340.3 (C-O-C ox. Str.), 1248.6(N-N ox), 1065.6(=C-O str.), 465 (C-Cl str.), MS (EI) m/z 334.98 [M+], Anal. Clad. For: $C_{14}H_7Cl_2N_3O_3$; C- 50.03, H- 2.1, N-12.5, Cl-21.09, O-14.28, Found: C-50.43, H-1.94, N-13.01, Cl-23.00, O-13.54%.

Antibacterial Activity

All the synthesized compounds were evaluated for their antibacterial potential by well diffusion assay[23] against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. These compounds were dissolved in DMSO to prepare different concentrations (50 and 100 µg/mL). On pre-inoculated plates with bacteria, wells were prepared in nutrient agar using micropipettes tips. In each well, 20 µL of the test sample of both concentrations were loaded along with standard antibiotic streptomycin. These plates were kept for incubation at 37°C for 24 hours. After that zone of inhibition for each compound was measured. The results obtained are shown in Table 4.

Antifungal Activity

The antifungal potential of all the synthesized compounds was evaluated using poison food approach.[24] First, a test organism is generated in a growth medium that contains the test substance. Next, the growth of the test organism is evaluated after it has been grown for some time and after exposure to the test substance. The experiments were carried out on the tested compounds to ascertain whether they successfully combat *Candida albicans* and *A. niger* strains. In addition to the three petri plates that held the solvents (control) and miconazole nitrate (as standard drug), three duplicates of each test item were utilized. Table 4 provides a simplified version of the findings from the tests that evaluated the substance's antifungal activity.

Result And Discussion

Chemistry

Consequently, a high yield (ranging from 60 to 95%) was accomplished in an appreciably shorter period (2–4 hours). Scheme 1 resulted in the production of a novel series of 2,5-substituted 1,3,4-oxadiazole derivatives. The process that may be engaged in the last stage of the synthesis of target molecules is depicted in Scheme 2, which illustrates one such procedure. The electron donation state may occur when groups that donate electrons, such as $-NH_2$ and -OH, are present. This might result in a decrease in the final compound yield, whereas the presence of electron-withdrawing groups such as $-NO_2$ and -Cl can bring about an increase in the final compound yield. It was discovered that the group of aryl acids known as the electron releasing group ($-OCH_3$, -OH) speed up the process more than the group known as the electron-withdrawing group did ($-NO_2$, -Cl). The synthesized compounds' physiochemical characteristics are summarized in Table 3, which may be found here (13-28). It was discovered that the overall yield of all the synthesized chemicals was between sixty and 95%. A TLC analysis was carried out to determine the purity level held by the synthesized compounds. FTIR, ^{1}H-NMR, and mass spectrometers were used to validate the synthesized compounds' structural properties. This was done to validate the compounds (MS).

By looking for a C=N stretching band in the range of 1455-1548 cm^{-1} in the infrared spectra of the produced compounds, it was feasible to locate the 1,3,4-oxadiazole ring. a C=O stretching band in the region of 1056–1070 cm^{-1}, and a C-O-C absorption band in the region of 1340–1450 cm^{-1}. These three bands are located at various wavelengths in the electromagnetic spectrum. These bands may all be found close to one another in their respective places. In the ^{1}H-NMR spectra of the newly synthesized compounds, peaks for -NH, -OH, and Ar-H could be found between the ranges of δ 3.9–5.0, 4.5–5.8, and 6.74–8.91, respectively. These ranges cover the newly synthesized compounds. The signals of the corresponding protons in the spectra displayed these peaks in their respective locations. When electron-withdrawing and electron-donating groups were switched out for the para, the relationship between the 1,3,4-oxadiazole nucleus and the phenyl ring, a difference was detected in the delta values of aromatic protons. The analysis of mass spectra offered further confirmation for the production method of 2,5-substituted diphenyl-1,3,4-oxadiazole, which offered more support for the creation of

Table 4: *In-vitro* antibacterial and antifungal activity of tested compounds (13-28)

Compound	*Zone of Inhibition (mm)*					
	Bacterial strains				*Fungal strains*	
	Staphylococcus aureus (Gram +vet)		*Escherichia coli (Gram –vet)*		*Aspergillus niger*	*Candida albicans*
Conc. (µg/mL)	*100*	*50*	*100*	*50*		
13	25	18	23	15	14	17
14	13	14	19	13	11	15
15	10	12	10	04	09	09
16	05	03	03	00	03	05
17	20	14	25	16	15	16
18	14	18	20	14	16	13
19	08	04	19	10	10	11
20	12	06	04	00	08	04
21	05	00	11	02	04	01
22	11	04	09	01	02	05
23	02	00	02	00	00	12
24	01	00	00	00	00	00
25	29	18	34	24	19	20
26	17	14	23	15	17	14
27	13	08	20	11	19	13
28	11	04	19	09	13	08
Streptomycin	48	20	50	25	-	-
Miconazole nitrate	-	-	-	-	20	24

the compound. Because each of the compound's M+1, M+2, and M+4 had a mass spectrum that comprised two atoms of chlorine, the chlorine patterns were very easy to see. The structure was validated by generating M+1 and M+2 chlorine patterns analogous.

Pharmacology

E. coli is a kind of bacterium frequently seen in the digestive tracts of humans. This bacterium can potentially cause secondary illnesses in addition to food poisoning. *S. aureus* has been linked to various illnesses, including pneumonia, staphylococcal endocarditis, and septic arthritis. Therefore, there is a good chance that these infections will harm people's health. This is a very real possibility. Therefore, numerous research has been conducted to evaluate the action against microorganisms demonstrated by the 1,3,4-oxadiazole the results of these experiments against S. *aureus* and E. *coli*. Investigations have offered favorable findings for the species above. As a direct consequence of this, it was believed that it would be useful to use these germs to investigate the effect of the antibiotic.

It was expected that many of the newly synthesized derivatives of 2,5-substituted diphenyl-1,3,4-oxadiazole (13-28) would display antibacterial activities, and as a result, research was carried out to test this idea. It was determined that streptomycin, a well-known antibiotic readily available for use in commercial settings, would serve as the standard for assessing antibacterial activity. According to the findings of the analyzed antibacterial screening, most of the compounds tested could inhibit the growth of bacteria in a manner that was detectable in a moderate to discernible manner. This was the conclusion drawn from the findings of the screening. The chemical 2-(4-chlorophenyl)-5-(2,4-chlorophenyl)-1,3,4-oxadiazole (25), which had the maximum activity when compared to all of the other compounds, was the most effective against S. *aureus* and E. *coli*; however, it was not as effective as the other compounds were against other bacteria. Compounds 13, 17, 18, and 26 only showed sporadic activity against E. *coli* and S. *aureus*, despite the activity indicated by the standard being much greater than that of those compounds. Compounds 23 and 24 exhibited antibacterial activity, although this activity was ineffective against all the bacteria examined. Antibacterial activity was found in all the remaining compounds, ranging from typical to extremely high levels.

Each of the compounds that were created underwent testing to see whether or not it has antifungal activity against *C. albicans* and A. *niger*. This was done by calculating the average zone of inhibition for each compound. The miconazole nitrate group served as the control in this research endeavor. Four molecules of 2-(4-chlorophenyl)-5-(4-methylphenyl)-1,3,4-oxadiazole (13), 2-(4-methylphenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (16), 2-(4-chlorophenyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (25) showed maximum activity against C. *albicans* although it was less effective than the standard. This was the case, although it was the compound with the highest efficacy. In a manner analogous to the previous example, the anti- *A. Niger* activity of compound 25 was shown to be the greatest. The remaining compounds exhibited antifungal activity ranging from moderate to poor when tested against the fungi investigated. Because of a choro substituent associated with the phenyl moiety related to the 1,3,4-oxadiazole ring, these compounds have a greater degree of activity compared to others with structures that are quite similar.

The findings of the experiments that measured antibacterial activity revealed that in the presence of an electron donor group (-OCH_3, -Cl, -OH, or -NH_2) biological activity was more intense than when an electron-withdrawing group was in the para position of the phenyl ring was present. This was the case regardless of the type of electron donor group (-NO_2). Therefore, compounds attached to the 2,5-position of the 1,3,4-oxadiazole ring and including dichlorvos and hydroxy substituents on the phenyl ring, have the potential to have increased antibacterial activities. Conversely, compounds containing nitro substituents on the para to the 1,3,4-oxadiazole ring has a probability of reducing antibacterial capabilities. Our studies are in accordance with a previous study which stated 1,3,4-oxadiazoles, a heterocyclic five-membered ring which plays a vital role in developing newer medicinal compounds for treating various biological activities, such as the proliferation of cells, tuberculosis, allergy, viral diseases.[25] Another study synthesized a series of novel 2,5-disubstituted-1,3,4-oxadiazoles from long-chain alkanoic and alkanoic acids. Those compounds were subjected to evaluation against some bacteria and fungi. In the study, these compounds were found to show good antimicrobial potential.[26]

Conclusion

A new category of 2,5-substituted diphenyl-1,3,4-oxadiazole derivatives have been developed and examined them to see their effectiveness as antibacterial agents against a particular group of microbes. The compounds had a lot of potentials. The whole group of newly produced compounds, including 2-(4-chlorophenyl) compounds-5-(4-nitrophenyl)-1,3,4-oxadiazole (13), 2-(4-methylphenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (16), 2-(4-chlorophenyl)-5-(4-methoxyhenyl)-1,3,4-oxadiazole (17), 4-[5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-yl]phenol (18), and 2-(4-chlorophenyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (25) have proven to have antibacterial and antifungal activity. Additional study is necessary to have a deeper comprehension of the molecular process that lies at the heart of the purported effect of these medications.

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