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Original article

SYNTHESIS OF SOME 2-*N*-PHENYLAMINO-5-(3,4-DICHLOROPHENYL)-1,3,4-OXADIAZOLE DERIVATIVES TOWARDS ANTIMICROBIAL ACTIVITY

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Abstract

Electrical energy offers numerous benefits for performing synthesis including increased reaction rates, yield enhancements, and cleaner chemistries. 2-N-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles were synthesized directly from the arylthiosemicarbazide at platinum electrode under controlled potential electrolysis in an undivided cell assembly in the acetonitrile. The synthesized compounds were screened for their in vitro growth inhibiting activity against different strains of bacteria and fungi viz., Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis and Staphylococcus aureus and antifungal activity against Aspergillus niger, Crysosporium pannical, Pellicularia solmanicolor and Candida albicans and results have been compared with the standard antibacterial Penicillin and antifungal Dithane-M 45. Compounds 1a, d, g and j exhibited better while 1b, e, f and k slightly less antibacterial activity than standard Penicillin. Compounds 1a, d and j exhibited better while 1b, e, i and k displayed slightly less antifungal activity than standard Dithane-M 45.

Key words: *Controlled potential, Electrolysis, Antibacterial activity, Antifungal activity, Platinum electrode.*

2N-Fenilamino-5-(3,4-diklorofenil)-1,3,4-oksadiazol Türevlerinin Sentezi ve Antimikrobiyal aktivitesi

Elektriksel enerjilerin reaksiyon hızının artması, verimin artmsası ve daha temiz kimyasal elde edilmesi gibi sentezin performansını arttıracak çok sayıda yazarı vardır. 2-N-fenilamino-5-(3,4-diklorofenil)-1,2,4-oksadiazoller bir bölünmemiş hücre içerisinde asetonitril ile birlikte kontrollü elektrolizi ile platin elektrot kullanılarak arilsemikarbazitden hareketle doğrudan sentez edilmiştir. Sentez edilen bileşikler in vitro büyümenin inhibisyonu yönünden Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Aspergillus niger, Crysosporium pannical, Pellicularia solmanicolor, Candida albicans gibi değişik bakteri ve mantar suşlarına karşı davranışları izlenmiştir ve sonuçlar standart antibakteriyel penisilin ve antifungal Dithan-M 45 ile elde edilenlerle karşılaştırılmıştır. 1a, d, o ve h bileşikleri standart penisilinden daha iyi bir antibakteriyel aktivite göstermesine karşılık, 1b, e, f ve k biraz daha az aktivite göstermiştir. 1a, d ve j bileşikleri standart Ditham-M 45 den daha iyi bir antifungal aktivite göstermesine karşılık 1b, e, i ve k biraz daha az aktivite göstermişlerdir.

Anahtar kelimeler: *Kontrollü potansiyel, Elektroliz, Antibakteriyel aktivite, Antifungal aktivite, Platin elektrot.*

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INTRODUCTION

Recent trends and advances in the technology for the development of ecofriendly synthetic method in the chemical research have great importance and is the need of the society. Today, the organic synthesis involving electrochemical techniques under suitable solvent and electrolytes are the basic requirements while multistep conventional synthesis produces considerable large amount of environmentally unfavorable wastes mainly due to a series of complex isolation procedure involving expensive and toxic solvents after each step. The electrochemical oxidation has various merits. These reactions do not require oxidizing reagents and can be performed at room temperature. Application of electricity as a non conventional energy source for activation of reactants in suitable solvents has now gained popularity over the usual homogeneous and heterogeneous reactions. It provides chemical processes with special attributes, such as enhanced reaction rate, higher yield of pure products, better selectivity and several ecofriendly advantages.

Many 1,3,4-oxadiazoles have been reported in the literature to have a broad spectrum of biological activity including anti-microbial (1,2), anti-fungal (3-5), anti-inflammatory (6,7) antitubercular (8), virucidal (9), antimalarial (10), analgesic (11), insecticidal (12) and herbicidal activity (13). During hit to lead efforts following a recent high throughput screening campaign, we initiated a program that required the synthesis of a series of 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles **1**. Literature synthesis of these oxadiazoles (14-20) include bromine oxidation of semicarbazide derivative and the cyclodesulfurization of acylthiosemicarbazide derivatives in the solution using I_2/NaOH or 1,3-dicyclohexylcarbodimide (DCC) (21-24), as well as mercury(II) acetate ($Hg(OAc)_2$) or yellow mercury(II) oxide HgO (25,26) and produce undesirable mercury byproducts that must than be removed and properly disposed off after the reaction is completed. These aforementioned solution phase methods, while successful, were deemed not readily amenable to high throughout synthesis, and thus did not meet our needs. The solution phase dehydrative synthesis from 1,2-diacylhydrazines and several solid-phase methods were also considered (27, 28). Evans (29) have synthesized similar cyclized product in one-pot preparation using resin-bound reagents. Electroorganic synthesis of 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles is the important step in this direction.

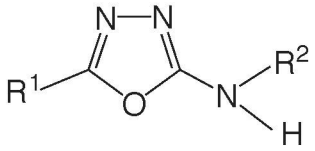

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Figure 1.2-N-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles

The main objective of this study was to find out a new simple synthetic method for the preparation of 1,3,4-oxadiazoles in which the use of aforementioned reagents could be minimized in amount and number and screen their in vitro growth inhibiting activity against different strains of bacteria and fungi. Here, we report that the electrooxidative cyclization of acylthiosemicarbazide **4** in acetonitrile affords 2-*N*-phenylamino-5-(4-nitrophenyl)-1,3,4-oxadiazoles in controlled potential electrolyses with a platinum plate anode at room temperature in an aprotic solvent acetonitrile.

MATERIALS AND METHODS

Apparatus

Melting points were recorded from open capillary and were uncorrected.

IR spectra in KBr were recorded on a Shimadzu 8201 PC IR spectrophotometer. ^{1}H-NMR (300, 300 MHz) and ^{13}C-NMR (75, 300 MHz) spectra were measured at room temperature on Bruker DRX 300 FT spectrometer instruments with TMS and $CDCl_3$ or C_6D_6 as internal standards. Carbon multiplities were assigned by DEPT techniques.

Microanalyses were carried out in the Elementar Vario EL III.

General procedure for the preparation of arylthiosemicarbazide (4a-l)

Arylisothiocyanate **3** was prepared directly from an aryl amine. The sparingly soluble ammonium aryldiathiacarbamate was obtained by the reaction of an arylamine, CS_2, and aqueous ammonia. Then aryldiathiacarbamate was decomposed by lead nitrate to produce arylisothiocyanate. The equimolar amount of arylhydrazine **2** and arylisothiocynate **3** were mixed in a small beaker with continuous stirring. After few minutes of stirring, the mixture was left overnight, which gave a solid compound arylthiosemicarbazide **4**.

Synthesis of 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles*: General procedure*

Thoroughly mixed arylthiosemicarbazide (18.0 mmol) and lithium perchlorate (3.0 mmol) in acetonitrile (200 mL) were taken in 250 mL three-electrode cell assembly with platinum plate (1.0 cm x 1.0 cm) as working as well as counter electrode and saturated calomel electrode (SCE) as reference electrode. Preparative-scale controlled potential electrolysis (30-34) were performed (25 ^{0}C) at their corresponding oxidation potential and completed in 3 to 5 h. The current potential data was recorded with potentiostat (Table 1). Magnetic stirrer was used for the diffusion of product from the electrode and proper mixing of reaction mixture. The products were extracted from the acetonitrile solution with chloroform by the simple solvent extraction and the extracted chloroform layer was separate out by rotatory evaporator. Purification by silica gel chromatography (benzene and methanol in 3 : 1) afforded **1** in excellent yield. All the synthesized compounds are new which are confirmed by characterization.

Table 1. Electrooxidative synthesis of 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles

Comp.	R^1 (-5)	R^2 (2-amino)	Time [h]	Applied Potential [V]	Current [mA]	Yield [%]
1a	3,4-$Cl_2C_6H_3$	C_6H_5	3	1.80	1180	83
1b	2,4-$Me_2C_6H_3$	C_6H_5	3	2.10	1016	76
1c	2,4,6-$(OMe)_3C_6H_2$	C_6H_5	4	1.95	1213	79
1d	3,4-$Cl_2C_6H_3$	2-$OMeC_6H_4$	5	2.00	1350	71
1e	2,4-$Me_2C_6H_3$	2-$OMeC_6H_4$	4	1.75	1055	81
1f	2,4,6-$(OMe)_3C_6H_2$	2-$OMeC_6H_4$	3	2.15	1115	84
1g	3,4-$Cl_2C_6H_3$	4-$OMeC_6H_4$	4	1.85	1185	78
1h	2,4-$Me_2C_6H_3$	4-$OMeC_6H_4$	5	1.90	1265	73
1i	2,4,6-$(OMe)_3C_6H_2$	4-$OMeC_6H_4$	4	2.10	1232	76
1j	3,4-$Cl_2C_6H_3$	2-MeC_6H_4	4	1.90	1205	83
1k	2,4-$Me_2C_6H_3$	2-MeC_6H_4	3	1.95	1015	81
1l	2,4,6-$(OMe)_3C_6H_2$	2-MeC_6H_4	4	2.05	1184	74

Analytical and spectral characterization

2-(N-Phenylamino)-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole (1a)

Brown crystals; m.p. 162-164 ^{0}C; IR (KBr) cm^{-1} 3230 (NH), 3045 (ArC-H), 1607 (C=N-N=C), 1265, 1069 (C-O-C), 910, 860, 735 (substituted benzene), 600-800 (Ar-Cl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25 (s, 1H, NH), 8.76 (s, 1H, ArH), 7.91 (d, 1H, J = 8.6 Hz, ArH), 7.75 (d, 1H, J = 8.6 Hz, ArH), 7.29-7.47 (m, 5H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.28 (oxadiazole-C_2), 159.85 (oxadiazole-C_5), 148.1 (arylamino-C_1), 139.8 (C_1), 129.8 (arylamino-C_3 and C_5), 135.1 (C_3), 129.4 (C_5), 127.1 (C_4), 125.7 (C_6), 124.9 (C_2), 119.0 (arylamino-C_4), 116.1 (arylamino-C_2 and C_6). MS (ESI) m/z Calcd $C_{14}H_9N_3OCl_2$ 307.15 (M + H), Found: 306.95. Anal. Calcd. C 54.87, H 2.94, N 13.72, Cl 22.86 % Found: C 54.11, H 2.54, N 13.42, Cl 22.49 %.

2-(N-Phenylamino)-5-(2,4-dimethylphenyl)-1,3,4-oxadiazole (1b)

Yellow crystals; m.p. 187-189 ^{0}C; IR (KBr) cm^{-1} 3250 (NH), 3045 (ArC-H), 2855 (aliphatic C-H), 1607 (C=N-N=C), 1265, 1069 (C-O-C), 910, 860, 735 (substituted benzene); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 8.78 (s, 1H, ArH), 7.89 (d, 1H, J = 8.6 Hz, ArH), 7.78 (d, 1H, J = 8.6 Hz, ArH), 7.28-7.99 (m, 5H, ArH), 2.28 (s, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.29 (oxadiazole C_2), 159.65 (oxadiazole C_5), 148.4 (arylamino-C_1), 138.90 (C_1), 136.5 (C_4), 135.9 (C_2), 129.7 (C_3), 128.8 (arylamino-C_3 and C_5), 126.3 (C_6), 126.1 (C_5), 119.12 (arylamino-C_4), 116.2 (arylamino-C_2 and C_6), 21.18 (CH_3). MS (ESI) m/z Calcd $C_{16}H_{15}N_3O$ 266.31 (M + H), Found: 265.04. Anal. Calcd. C 72.37, H 5.65, N 15.83 %, Found: C 71.85, H 5.17, N 15.23 %.

2-(N-Phenylamino)-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole (1c)

Brown needles; m.p. 201-203 ^{0}C; IR (KBr) cm^{-1} 3260 (NH), 3055 (ArC-H), 2855 (aliphatic C-H), 1604 (C=N-N=C), 1255, 1075 (C-O-C), 950, 860, 740 (substituted benzene); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.32 (s, 1H, NH), 6.97-7.88 (m, 5H, ArH), 6.24 (s, 2H, ArH_3 and H_5), 3.78 (s, 3H, 4-OCH_3), 3.70 (s, 6H, 2,6-OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.48 (oxadiazole C_2), 160.8 (C_6), 160.3 (C_4), 160.3 (C_2), 157.45 (oxadiazole C_5), 148.4 (arylamino-C_1), 128.8 (arylamino-C_3 and C_5), 119.12 (arylamino-C_4), 116.2 (arylamino-C_2 and C_6), 104.3 (C_1), 91.9 (C_3), 91.4 (C_5), 55.9 (2,6-OCH_3), 55.4 (4-OCH_3). MS (ESI) m/z Calcd $C_{17}H_{17}N_3O_4$ 328.34 (M + H), Found: 327.96. Anal. Calcd. C 62.32, H 5.19, N 12.83 %, Found: C 61.92, H 5.03, N 12.63 %.

2-[N-(2-Methoxyphenyl)amino]-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole (1d)

Dark brownish needles; m.p. 177-179 ^{0}C; IR (KBr) cm^{-1} 3244 (NH), 2927 (ArC-H), 2822 (O-CH_3), 2853 (aliphatic C-H), 1611 (C=N-N=C), 1250, 1062 (C-O-C), 915, 870, 675 (substituted benzene), 600-800 (Ar-Cl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 8.77 (s, 1H, ArH), 7.89 (d, 1H, J = 8.6 Hz, ArH), 7.79 (d, 1H, J = 8.6 Hz, ArH), 6.92-7.45 (m, 4H, ArH), 3.74 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.75 (oxadiazole C_2), 158.47 (oxadiazole C_5), 147.5 (arylamino-C_2), 140.8 (C_1), 135.6 (C_3), 133.5 (arylamino-C_1), 129.4 (C_5), 127.9 (C_4), 125.6 (C_6), 124.3 (C_2), 122.1 (arylamino-C_5), 119.9 (arylamino-C_4), 117.1 (arylamino-C_6), 115.4 (arylamino-C_3), 53.7 (OCH_3). MS (ESI) m/z Calcd $C_{15}H_{11}N_3O_2Cl_2$ 337.17 (M + H), Found: 336.84. Anal. Calcd. C 53.56, H 3.27, N 12.50, Cl 21.12 %, Found: C 53.06, H 3.12, N 12.15, Cl 20.89 %.

2-[N-(2-Methoxyphenyl)amino]-5-(2,4-dimethylphenyl)-1,3,4-oxadiazole (1e)

Dark brown crystals; m.p. 195-197 ^{0}C; IR (KBr) cm^{-1} 3261 (NH), 3045 (ArC-H), 2855 (aliphatic C-H), 2815 (OCH_3), 1609 (C=N-N=C), 1270, 1071 (C-O-C), 915, 870, 790 (substituted benzene); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 8.78 (s, 1H, ArH), 7.89 (d, 1H, J = 8.6 Hz, ArH), 6.93-7.03 (m, 4H, ArH), 7.78 (d, 1H, J = 8.6 Hz, ArH), 3.11 (s,

3H, OCH_3), 2.21 (s, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.28 (oxadiazole C_2), 159.85 (oxadiazole C_5), 147.5 (arylamino-C_2), 138.78 (C_1), 136.49 (C_4), 135.9 (C_2), 133.5 (arylamino-C_1), 129.42 (C_3), 126.5 (C_6), 126.2 (C_5), 122.3 (arylamino-C_5), 119.3 (arylamino-C_4), 117.6 (arylamino-C_6), 115.1 (arylamino-C_3), 53.8 (OCH_3), 21.9 (CH_3). MS (ESI) m/z Calcd $C_{17}H_{17}N_3O_2$ 296.34 (M + H), Found: 296.00. Anal. Calcd. C 69.07, H 5.75, N 14.22 %, Found: C 68.87, H 5.42, N 14.85 %.

2-[N-(2-Methoxyphenyl)amino]-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole (1f)

Dark brown crystals; m.p. 195-197 ^0C; IR (KBr) cm^{-1} 3260 (NH), 3043 (ArC-H), 2860 (aliphatic C-H), 2822 (O-CH_3), 1608 (C=N-N=C), 1280, 1066 (C-O-C), 925, 890, 785 (substituted benzenes); ^1H NMR (300 MHz, DMSO-d_6): δ 10.29 (s, 1H, NH), 6.89-7.75 (m, 4H, ArH), 6.24 (s, 2H, ArH_3 and H_5), 3.78 (s, 3H, OCH_3), 3.76 (s, 3H, 4-OCH_3), 3.70 (s, 6H, 2,6-OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.48 (oxadiazole C_2), 160.8 (C_6), 160.3 (C_4), 160.2 (C_2), 157.45 (oxadiazole C_5), 147.5 (arylamino-C_2), 133.5 (arylamino-C_1), 122.1 (arylamino-C_5), 119.9 (arylamino-C_4), 117.1 (arylamino-C_6), 115.4 (arylamino-C_3), 104.3 (C_1), 91.9 (C_3), 91.4 (C_5), 55.9 (2,6-OCH_3), 55.4 (4-OCH_3), 53.8 (OCH_3). MS (ESI) m/z Calcd $C_{18}H_{19}N_3O_5$ 359.35 (M + H), Found: 358.78. Anal. Calcd. C 60.27, H 5.30, N 11.72 %, Found: C 59.68, H 5.12, N 11.45 %.

2-[N-(4-Methoxyphenyl)amino]-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole (1g)

Yellow crystals; m.p. 176-178 ^0C; IR (KBr) cm^{-1} 3236 (NH), 3033 (=C-H), 3030 (ArC-H), 2855 (aliphatic C-H), 2820 (O-CH_3), 1670 (C=C), 1606 (C=N-N=C), 1262, 1067 (C-O-C), 920, 860, 790 (substituted benzenes), 600-800 (Ar-Cl); ^1H NMR (300 MHz, DMSO-d_6): δ 10.32 (s, 1H, NH), 8.76 (s, 1H, ArH), 7.91 (d, 1H, J = 8.6 Hz, ArH), 7.75 (d, 1H, J = 8.6 Hz, ArH), 7.71 (d, 2H, J = 8.6 Hz, ArH), 7.62 (d, 2H, J = 8.6 Hz, ArH), 3.77 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.08 (oxadiazole C_2), 157.45 (oxadiazole C_5), 139.6 (C_1), 135.6 (C_3), 132.6 (arylamino-C_1), 129.2 (C_5), 127.8 (C_4), 127.5 (arylamino-C_2 and C_6), 125.8 (C_6), 124.5 (C_2), 119.5 (arylamino-C_4), 114.1 (arylamino-C_3 and C_5), 55.5 (OCH_3). MS (ESI) m/z Calcd $C_{15}H_{11}N_3O_2Cl_2$ 337.17 (M + H), Found: 336.84. Anal. Calcd. C 53.55, H 3.27, N 12.50, Cl 21.76 %, Found: C 53.11, H 3.03, N 12.12, Cl 21.36 %.

2-[N-(4-Methoxyphenyl)amino]-5-(2,4-dimethylphenyl)-1,3,4-oxadiazole (1h)

Brownish needles; m.p. 174-176 ^0C; IR (KBr) cm^{-1} 3244 (NH), 2927 (ArC-H), 2870 (aliphatic C-H), 2822 (O-CH_3), 1611 (C=N-N=C), 1250, 1063 (C-O-C), 915, 870, 675 (substituted benzene); ^1H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 8.78 (s, 1H, ArH), 7.89 (d, 1H, J = 8.6 Hz, ArH), 7.79 (d, 1H, J = 8.6 Hz, ArH), 7.71 (d, 2H, J = 8.6 Hz, ArH), 7.63 (d, 2H, J = 8.6 Hz, ArH), 3.74 (s, 3H, OCH_3), 2.28 (s, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.75 (oxadiazole C_2), 158.47 (oxadiazole C_5), 158.3 (arylamino-C_4), 138.5 (C_1), 136.5 (C_4), 135.9 (C_2), 132.5 (arylamino-C_1), 129.8 (C_3), 127.5 (arylamino-C_2 and C_6), 126.8 (C_5), 126.6 (C_6), 114.1 (arylamino-C_3 and C_5), 55.3 (OCH_3), 21.2 (CH_3). MS (ESI) m/z Calcd $C_{17}H_{17}N_3O_2$ 296.34 (M + H), Found: 296.11. Anal. Calcd. C 69.07, H 5.75, N 14.22 %, Found: C 68.41, H 5.56, N 14.05%.

2-[N-(4-Methoxyphenyl)amino]-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole (1i)

Brownish needles; m.p. 185-187 ^0C; IR (KBr) cm^{-1} 3244 (NH), 2927 (ArC-H), 2870 (aliphatic C-H), 2815 (O-CH_3), 1616 (C=N-N=C), 1250, 1065 (C-O-C), 915, 870, 675 (substituted benzene); ^1H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 8.24 (s, 2H, ArH), 7.79 (d, 2H, J = 8.6 Hz, ArH), 7.71 (d, 2H, J = 8.6 Hz, ArH), 3.78 (s, 3H, 4-OCH_3), 3.75 (s, 3H, OCH_3), 3.70 (s, 6H, 2,6-OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.36 (oxadiazole C_2), 160.8 (C_6), 160.5 (C_4), 160.2 (C_2), 158.47 (oxadiazole C_5), 141.3 (arylamino-C_1), 136.0

(arylamino-C_2), 129.3 (arylamino-C_3), 126.8 (arylamino-C_4), 126.7 (arylamino-C_6), 125.8 (arylamino-C_5), 104.2 (C_1), 91.9 (C_3), 91.4 (C_5), 55.2 (4-OCH_3), 54.9 (2,6-OCH_3). MS (ESI) m/z Calcd $C_{18}H_{19}N_3O_5$ 359.35 (M + H), Found: 358.71. Anal. Calcd. C 60.29, H 5.30, N 11.72 %, Found: C 59.67, H 5.06, N 11.25 %.

2-[N-(2-Methylphenyl)amino]-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole (1j)

Brownish needles; m.p. 179-181 ^{0}C; IR (KBr) cm^{-1} 3244 (NH), 2927 (ArC-H), 2870 (aliphatic C-H), 1611 (C=N-N=C), 1250, 1067 (C-O-C), 915, 870, 675 (substituted benzene), 600-800 (ArCl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.36 (s, 1H, NH), 8.75 (s, 1H, ArH), 7.91 (d, 1H, *J* = 8.6 Hz, ArH), 7.75 (d, 1H, *J* = 8.6 Hz, ArH), 7.35-7.45 (m, 4H, ArH), 1.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.72 (oxadiazole C_2), 158.47 (oxadiazole C_5), 141.3 (arylamino-C_1), 140.1 (C_1), 136.10 (arylamino-C_2), 135.6 (C_3), 129.4 (C_5), 129.3 (arylamino-C_3), 127.5 (C_4), 126.8 (arylamino-C_4), 126.7 (arylamino-C_6), 125.8 (arylamino-C_5), 125.6 (C_6), 124.9 (C_2), 21.18 (CH_3). MS (ESI) m/z Calcd $C_{15}H_{11}N_3OCl_2$ 321.18 (M + H), Found: 320.89. Anal. Calcd. C 56.24, H 3.43, N 13.12, Cl 22.18 %, Found: C 55.98, H 3.15, N 13.02, Cl 22.03 %.

2-[N-(2-Methylphenyl)amino]-5-(2,4-dimethylphenyl)-1,3,4-oxadiazole (1k)

Yellow crystals; m.p. 190-192 ^{0}C; IR (KBr) cm^{-1} 3261 (NH), 3045 (ArC-H), 3033 (=C-H), 2855 (aliphatic C-H), 1609 (C=N-N=C), 1270, 1069 (C-O-C), 915, 870, 790 (substituted benzene); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.55 (s, 1H, NH), 8.78 (s, 1H, ArH), 7.89 (d, 1H, *J* = 8.6 Hz, ArH), 7.79 (d, 1H, *J* = 8.6 Hz, ArH), 7.35-7.45 (m, 4H, ArH), 1.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.25 (oxadiazole C_2), 158.47 (oxadiazole C_5), 141.5 (arylamino-C_1), 138.78 (C_1), 136.49 (C_4), 136.0 (arylamino-C_2), 135.42 (C_2), 129.42 (C_3), 129.3 (arylamino-C_3), 126.8 (arylamino-C_4), 126.7 (arylamino-C_6), 126.5 (C_6), 126.2 (C_5), 125.8 (arylamino-C_5), 21.50 and 21.18 (CH_3). MS (ESI) m/z Calcd $C_{17}H_{17}N_3O$ 280.34 (M + H), Found: 279.95. Anal. Calcd. C 72.77, H 6.08, N 15.03 %, Found: C 71.64, H 5.82, N 14.57 %.

2-[N-(2-Methylphenyl)amino]-5-(2,4,6-trimethoxyphenyl)-1,3,4-oxadiazole (1l)

Brownish needles; m.p. 193-195 ^{0}C; IR (KBr) cm^{-1} 3244 (NH), 2927 (ArC-H), 2870 (aliphatic C-H), 2822 (OCH_3), 1611 (C=N-N=C), 1585 (Ar-NO_2), 1250, 1061 (C-O-C), 915, 870, 675 (substituted benzene); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 8.24 (s, 2H, ArH), 7.35-7.46 (m, 4H, ArH), 3.78 (s, 3H, 4-OCH_3), 3.70 (s, 6H, 2,6-OCH_3), 1.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.12 (oxadiazole C_2), 160.5 (C_4), 160.3 (C_2), 160.3 (C_6), 158.47 (oxadiazole C_5), 141.3 (arylamino-C_1), 136.1 (arylamino-C_2), 129.3 (arylamino-C_3), 126.8 (arylamino-C_4), 126.7 (arylamino-C_6), 125.8 (arylamino-C_5), 104.9 (C_1), 91.7 (C_3), 91.4 (C_5), 55.4 and 54.8 (OCH_3), 21.18 (CH_3). MS (ESI) m/z Calcd $C_{18}H_{19}N_3O_4$ 342.39 (M + H), Found: 341.01. Anal. Calcd. C 63.27, H 5.56, N 12.30%, Found: C 63.06, H 5.32, N 11.85 %.

Screening for Antimicrobial activity

Antibacterial and antifungal tests

The MIC determination of the tested compounds was carried out in side-by-side comparison with the reference drugs Penicillin for antibacterial activity and Dithane-M 45 for antifungal activity by experimental method of Benson (35). Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 μg/mL. The tubes were Inoculated with 10^5 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ^{0}C for 18 h. The lowest concentration, which showed no visible growth on the plate, was taken as an end point minimum inhibitory concentration (MIC).

To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The MIC levels of compounds against the organisms are given in Table 2 and Table 3.

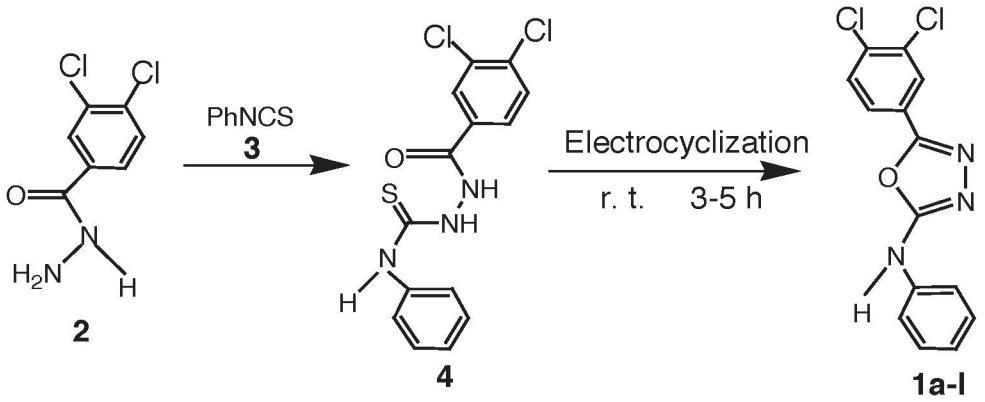
Microorganisms

Standard strains of the following bacteria, namely *Klebsiella pneumoniae* (ATCC 1003), *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 60511) and *Staphylococcus aureus* (ATCC 11632) for the determination of antibacterial activity, and standard strains of *Aspergillus niger* (ATCC 16404), *Crysosporium pannical* (ATCC 10231), *Pellicularia solmanicolor* (ATCC 97556) and *Candida albicans* (ATCC 10231) for the determination of antifungal activity were used. All the bacterial and fungal isolates were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratories, Pune, India.

RESULTS AND DISCUSSION

Chemistry

Our objective was to find out a new general environmentally benign synthetic method for the preparation of 1,3,4-oxadiazoles in which the use of aforementioned reagents could be minimized by amount and number both as well as test newly synthesized compounds against different strains of bacteria and fungi. Keeping this objective in mind we have synthesized some 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles **1** by electrooxidative cyclization of arylthiosemicarbazide **4** at the platinum electrode. This electrochemical cyclization gives the oxadiazoles (Scheme 1 and Scheme 2) without requirement of any hazardous reagents. We used acetonitrile as a solvent and lithium perchlorate ($LiClO_4$) as an electrolyte that can be handled very easily without major precautions.



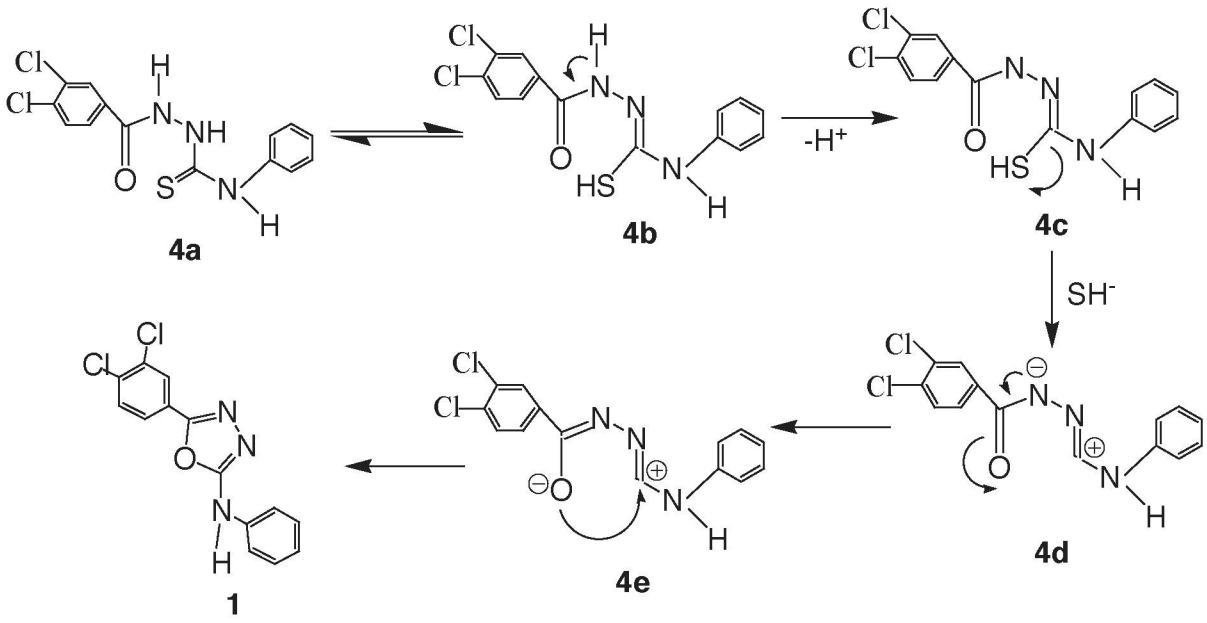
Scheme 1. Synthesis of 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles

In the IR spectrum of **2** broad stretching bands at around 3337 cm^{-1} and 3278 cm^{-1} were due to amine/amide NH while strong stretching band at 1615 cm^{-1} was attributed to amide carbonyl. ^1H NMR spectrum showed a singlet at δ 4.51 and δ 9.81 which were accounted for NH_2 and NH which vanished on D_2O exchange. Two protons of phenyl moiety resonated as two doublets at δ 7.68 and δ 7.90 and one proton at singlet δ 8.60. The mass spectrum of **2a** showed a molecular ion peak at m/z 205 which confirmed its molecular weight.

In the IR spectrum of **4** broad stretching bands at around 1632 cm^{-1} for carbonyl and 1265 - cm^{-1} for C=S bonding. ^1H NMR spectrum showed a singlet at δ 10.60-11.96 which were accounted for NH which vanished on D_2O exchange. Two protons of phenyl moiety resonated

as two doublets at δ 7.32 and δ 7.55 and one proton as a singlet at δ 8.61. Five protons show multiplet at δ 6.95-7.46. The mass spectrum of **4** showed a molecular ion peak at m/z 340 which confirmed its molecular weight.

Lack of ^{1}H NMR resonances observed with NH and NH_2 functions in the ^{1}H NMR spectrum of **1** proved that ring closure starting from **4** resulted in the formation of 1,3,4-oxadiazole ring. This was further substantiated by the ^{13}C NMR data of **1** which showed a peak δ 174-177 and 157-160 due to C_2 and C_5 of oxadiazole. The IR spectrum shows 1604-1616 cm^{-1} for (C=N-N=C) and 1062-1075 cm^{-1} for (C-O-C) in the compounds **1a-l** which confirmed the synthesis of 1,3,4-oxadiazoles.



Scheme 2. Mechanistic proposal

Antimicrobial activity

The compounds were tested for *in vitro* activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The antifungal activities of compounds were evaluated against *Aspergillus niger*, *Crysosporium pannical*, *Pellicularia solmanicolor* and *Candida albicans*. Penicillin was used as positive control against bacteria and Dithane-M 45 against fungi. The compounds inhibited growth of the bacteria and fungi with MICs between 8 to 500 μg/mL.

Antibacterial activity indicates that compounds **1a, d, g** and **j** are found to be most active against *Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* organisms taking Penicillin as the standard. The majority of the compounds exhibited antibacterial activity against *E. coli, K. pneumoniae, B. subtilis* and *S. aureus* as compared to standard Penicillin. Compound **1c** have moderate activity against S. aureus. Compound **1k** displayed the antibacterial activity in moderate range against all the strains. Compound **1h, i** and **l** exhibited weak antibacterial activity against all bacterial strains used for our evaluation.

The screening results showed that compounds **1a, d** and **j** displayed good antifungal activity against all antifungal strains in compared with the standard fungicide Diethane-M 45. The compounds **1b, e** and **g** showed antifungal activity in the moderate range. The compounds **1c, f, h, i** and **l** displayed very weak or negligible in comparison to Dithane-M 45.

The antimicrobial activity of the compounds varied upon the type and position of the substituents at 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles moiety. It can be concluded from the antimicrobial screening results that when 2-*N*-phenylamino-5-(3,4-dichlorophenyl)-1,3,4-oxadiazoles were substituted with aryl halide the antimicrobial activity was altered to an appreciable extent.

Table 2. Antibacterial activity of compounds **1a-1l**

Compound	Minimum inhibitory concentration (MIC) (µg/mL)			
	E. coli	*K. pneumoniae*	*B. subtilis*	*S. aureus*
1a	8	31.25	8	8
1b	62.5	62.5	62.5	500
1c	250	125	500	31.25
1d	8	31.25	8	31.25
1e	62.5	31.25	31.25	31.25
1f	125	62.5	250	125
1g	8	31.25	31.25	8
1h	500	500	500	250
1i	500	500	500	500
1j	8	8	8	31.25
1k	62.5	62.5	31.25	62.5
1l	500	500	500	500
Penicillin	≤ 8	≤ 8	≤ 8	≤ 8

DMSO was used as a control which has no activity

Table 3. Antifungal activity of the compounds **1a-1l**

Compound	Minimum inhibitory concentration (MIC) (µg/mL)			
	A. niger	*P. solmanicolor*	*C. pannical*	*C. albicans*
1a	4	4	31.25	4
1b	16	31.25	62.5	31.25
1c	500	500	500	500
1d	4	4	16	4
1e	16	62.5	125	31.25
1f	500	500	500	500
1g	16	31.25	62.5	125
1h	500	500	500	500
1i	250	500	125	250
1j	8	31.25	16	16
1k	16	250	250	125
1l	500	500	500	500
Dithane-M 45	8	≥ 8	≤ 8	≥ 8

DMSO was used as a control which has no activity

CONCLUSION

It is evident from the electrochemical method that the electroorganic synthesis of 1,3,4-oxadiazole derivatives is an example of electrochemical cyclization by electrooxidation of arylthiosemicarbazide. It provides a good method for the synthesis of oxadiazoles, in excellent yields. In the present electrolytic method, electrolysis was carried out at ordinary temperature and no hazardous chemicals were used. Therefore the method is environmentally benign and the great contribution in the field of green chemistry. The compounds containing chloro substituent at the 5-aryl position are most important antimicrobial agents.

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