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MICROWAVE ASSISTED SYNTHESIS AND PHARMACOLOGICAL STUDIES OF SOME NOVEL 1,3,4-OXADIAZOLE DERIVATIVES POSSESSING 1,2,3-TRIAZOLE

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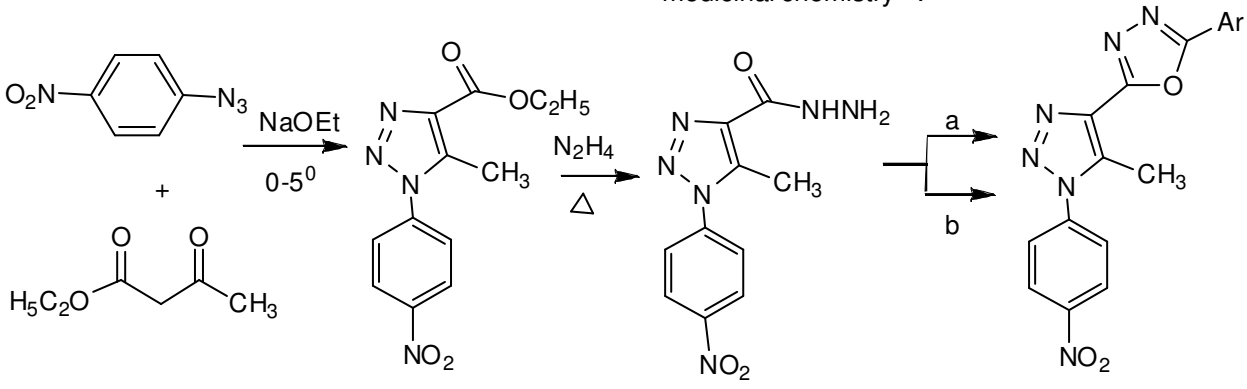
A novel series of 2-[5-methyl-1-(*p*-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-aryl-1,3,4-oxadiazoles (**3**), was prepared from 5-methyl-1-(*p*-nitrophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (**2**) by following both conventional and microwave mediated eco friendly approach. The structures of new oxadiazole derivatives (**3**) were characterized on the basis of IR, NMR, mass spectral data and elemental analysis. The newly synthesized compounds were screened for their antimicrobial activity by well plate method (zone of inhibition). Antioxidant studies of the synthesized compounds were also performed by measuring the DPPH radical scavenging assay.

1,3,4-Oxadiazole is a versatile lead molecule for designing potential bioactive agents. The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial[1], analgesic[2], antiinflammatory[3] and other biological properties such as genotoxic studies[4] and lipid peroxidation inhibitor[5].

1,2,3-Triazoles are attractive constructs, which because of their unique chemical properties and structure find many applications in organic and medicinal chemistry[6,7].



Ar = 1-(4-isobutylphenyl) ethyl, C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 2, 4-$Cl_2C_6H_3$, 2-Cl,4-FC_6H_3

(a) $POCl_3$, MWI (b) $POCl_3$, reflux 18 hr.

SCHEME-1

Table-1
Characterization data of 2-[5-methyl-1-(nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-aryl-1,3,4-oxadiazoles (**3**a-h)

Comp	Ar	M.P. (^{0}C)	conventional Yield (%)	MW Yield (%)
3a[b]	Isobutyl phenylethyl	112-114	66	86
3b[a]	C_6H_5	238-240	70	82
3c[a]	4-$CH_3C_6H_4$	180-184	76	80
3d[b]	4-$OCH_3C_6H_4$	178-180	75	76
3e[a]	4-ClC_6H_4	220-224	70	70
3f[a]	4-BrC_6H_4	218-220	70	76
3g[b]	2,4-$Cl_2C_6H_3$	150-152	65	75
3h[b]	2-Cl,4-F-C_6H_3	210-214	68	70

Solvent for recrystallisation : [a]Ethanol; [b]Ethanol + DMF.

The design of new compounds and development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased biological activity. These observations prompted us to synthesize new 1,3,4-oxadiazole derivatives carrying 1,2,3-triazole moiety. Both 1,3,4-oxadiazoles and 1,2,3-triazoles are two important pharmacophores so that when they combine they may result in the formation of potent bio-active molecules. Prompted by these observations and in continuation of our effort in developing eco-friendly methods in the synthesis of bioactive molecules[8-11], we synthesized the target molecules and evaluated for their antimicrobial and antioxidant study.

The structures and characterization data of 2-(substituted)-5-[5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazoles (**3**a-h) were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. Characterization data of all the newly synthesized compounds are presented in Table-1.

In the IR spectra of 2-(substituted)-5-[5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazoles (**3**a-h) the disappearance of carbonyl stretching band clearly showed the formation of oxadiazole from the corresponding hydrazide.

In the ^{1}H NMR spectrum of 2-(substituted)-5-[5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazoles (**3**a-h) the signal due to methyl group of 1,2,3-triazole appeared as a singlet at δ 2.45-2.77 ppm, aromatic protons of 4-nitrophenyl appeared as two doublets in the region 7.90-8.25 ppm and 8.51-8.64 ppm, integrating for two protons each, the disappearance of NH_2 and NH proton clearly showed the formation of oxadiazole derivative from the corresponding hydrazide.

Pharmacology

Some of the selected compounds from this series were evaluated for antibacterial and antioxidant activity. The antibacterial activity was carried out using well plate technique. The antioxidant activity was carried out using DPPH radical scavenging assay.

Antibacterial activity

The antibacterial activity of the synthesized compounds **3**a-h was done using *Bacillus subtilis* MTCC 441, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The above activity was examined qualitatively and quantitatively by the presence or absence of inhibition zones and zone diameter. Susceptibility of the test organism to the organic compound was determined by well plate technique. Each strain was inoculated into 10 ml Tryptone Soya Broth (TSB) in 50 ml conical flask and was incubated at 37^0 till they showed good growth. From the well grown flask 60 µl of the inoculum was spread uniformly on the pre-set media plates. The

Table-2
Antibacterial activity of compounds **3**a-**3**h (Zone of inhibition in mm)

Compd	*E. coli*		*B. subtilis*		*P. aeruginosa*	
Concentration in mg/ml	1	0.5	1	0.5	1	0.5
Streptomycin	17 ± 0.2	15 ± 0.1	20 ± 0.3	17 ± 0.2	16 ± 0.1	13 ± 0.1
Control	00	00	00	00	00	00
3a	06 ± 0.0	04 ± 0.2	05 ± 0.3	03 ± 0.2	05 ± 0.2	03 ± 0.3
3b	10 ± 0.2	07 ± 0.3	12 ± 0.2	10 ± 0.3	11 ± 0.2	08 ± 0.1
3c	13 ± 0.1	10 ± 0.2	12 ± 0.2	10 ± 0.1	14 ± 0.2	12 ± 0.1
3d	13 ± 0.2	11 ± 0.2	14 ± 0.3	11 ± 0.2	14 ± 0.3	12 ± 0.2
3e	06 ± 0.3	03 ± 0.2	07 ± 0.2	05 ± 0.1	06 ± 0.2	04 ± 0.3
3h	06 ± 0.3	03 ± 0.2	07 ± 0.2	05 ± 0.1	06 ± 0.2	04 ± 0.3

wells were dug by sterilized cork borer and organic compound dissolved in DMSO (1mg/ml and 0.5mg/ml concentration) were added. Same procedure was repeated for all micro-organisms, the petri plates were incubated for 24 hr at 37^0. Here dimethyl sulfoxide (DMSO) was used as negative control and streptomycin as positive controls. The plates were checked for zone of inhibition, the compounds which showed good zone inhibition, were studied for minimum inhibitory concentration (MIC). MIC was performed at different concentration 8, 16, 35, 125, 250, 500 and 1000 µg/ml. 100 µl of the inoculum was uniformly spread onto pre-set plates and then placed sterile filter paper disks (5 mm diameter) on the spread plates. The filter paper disk was loaded with 5 µl of the sample of different concentration before starting the experiment aseptically. The plates were incubated at 37^0 for 24 hr. The antibacterial activity results are tabulated in Table-2.

Antioxidant activity

Free radical scavenging activity of the test compounds (**3**a-h) were carried out based on the scavenging activity of stable DPPH. 100 µg/ml of each test sample and standard BHT was taken in different test tubes and the volume was adjusted to 1 ml using MeOH.Freshly prepared 3 ml of 0.1 mM DPPH solution was mixed and vortexed thoroughly and left in dark for 30 min. The absorbance of stable DPPH radical was measured at 517 nm. The DPPH control (containing no sample) was prepared using the same procedure. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{(\text{Abs Control})} \times 100$$

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + test sample/standard BHT. The antioxidant study results are tabulated in Table-3.

Among the compounds tested for antibacterial activity **3**b, **3**c and **3**d showed activity comparable with that of the standard drug streptomycin. Among the compounds screened for DPPH radical scavenging

Table-3
DPPH radical scavenging activity of compounds **3**a-**3**h

Compd	DPPH assay in %
3a	68.36
3b	55.91
3c	70.60
3d	73.12
3e	70.84
3h	40.50
BHT	90.42

activity, compounds **3**c, **3**d and **3**e showed comparable activity with BHT. It is interesting to note that **3**d and **3**c showed good antibacterial and antioxidant activity comparable to that of standard, it clearly concluded that presence of electron releasing group in the aryl group will enhance the biological activity. Similarly the results showed that microwave induced reactions were found to be more efficient than the conventional method.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on a Shimadzu FT-IR Prestige-21 spectrophotometer. ^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer and all the chemical shift values were reported in δ scale. Mass spectra were recorded on a Waters UPLC-MS or API 3000 LC-MS spectrometer. Microwave reactions were performed on a Catalyst systems CATA R microwave synthesizer.

Ethyl 5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylate 1 : General procedure

1-Azido-4-nitrobenzene (0.01 mol) was treated with ethyl acetoacetate (0.01 mol) in methanol (75 ml) and the mixture was cooled to 0^0. Sodium ethoxide (0.01 mol) was added under inert atmosphere to the above mixture and stirred at ambient temperature for 8 hr. Progress of the reaction was monitored by TLC. The precipitated solid was filtered, washed with water and recrystallized from ethanol and structure was confirmed by single crystal XRD study[11], yield 75%, m.p. 165-170^0. [Found : C, 52.14, H, 4.34, N, 20.28 $C_{12}H_{12}N_4O_4$ requires C, 52.17, H, 4.38, N, 20.28%].

5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide 2 : General procedure

Ethyl-5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylate (0.05 mol) in DMF and hydrazine hydrate (99%, 0.05 mol) were taken in a round bottomed flask equipped with reflux condenser. The contents were refluxed for 6 hr. Precipitate of 2-carbethoxy-4-nitrophenylhydrazine was filtered, dried and recrystallized using ethanol. Yield 80%, m.p. 210-214^0. [Found : C, 45.82, H, 3.86, N, 32.06 $C_{10}H_{10}N_6O_3$ requires C, 45.80, H, 3.84, N, 32.05%].

2-Substituted-5-[5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3,4-oxadiazols 3a-h : General procedure

An equimolar mixture of 5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (**2**) (0.005 mol) and appropriate acids (**2**) (0.005 mol) were dissolved in phosphorus oxychloride. This mixture was then heated conventionally for about 18 hr or by employing microwave irradiation for about 5 minutes at 210 watts. The reaction mixture was then cooled to room temperature and poured into ice cold water and treated with saturated solution of sodium bicarbonate. It was then filtered, dried and recrystallized from ethanol. The characterization data of these compounds are given in Table-1.

2-[5-Methyl-1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl]-5-[1-(4-isobutylphenyl) ethyl]-1,3,4-oxadiazole 3a

IR (KBr):cm^{-1}: 2954 (C-H), 1593 (C=N), 1521 (asym, NO_2), 1344 (sym. NO_2); ^{1}H NMR (DMSO): δ

0.85 (d, 6H, *J*=6.6 Hz, $(CH_3)_2$), 1.34 (d, 3H, *J*=7.12 Hz, CH-CH_3), 1.70-1.81 (m, 1H, $CH(CH_3)_2$), 2.43 (d, 2H, *J*=7.08 Hz, CH_2), 2.66 (s, 3H, CH_3), 4.64 (q, 1H, CH), 7.17 (d, 2H, *J*=7.92 Hz, 3′,5′-Ib-ArH), 7.27 (d, 2H, *J*=8.04 Hz, 2′,6′-Ib-ArH), 8.03 (d, 2H, *J*=8.96 Hz, meta protons of *p*-nitrophenyl), 8.51 (d, 2H, *J*=9.0 Hz, ortho protons of *p*-nitrophenyl); LC-MS (m/z): 433 [M^++1].

2-[5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-phenyl-1,3,4-oxadiazole 3b

IR (KBr): 3005 (C-H), 1593 (C=N), 1543 (asym. NO_2), 1396 (sym. NO_2); ^{1}H NMR (DMSO): 2.62 (s, 3H, CH_3), 7.25-7.40 (m, 5H, ArH), 7.90 (d, 2H, *J*=8.9 Hz, meta protons of *p*-nitrophenyl), 8.52 (d, 2H, *J*=8.9 Hz, ortho protons of *p*-nitrophenyl); UPLC-MS (m/z): 349 (M^++1).

2-[5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-methylphenyl)-1,3,4-oxadiazole 3c

IR (KBr): 2960 (C-H), 1568 (C=N), 1510 (asym. NO_2), 1372 (sym. NO_2); ^{1}H NMR (DMSO): 2.45 (s, 3H, triazole CH_3), 2.62 (s, 3H, CH_3), 7.02 (d, 2H, *J*=8.68 Hz, ortho protons of *p*-tolyl), 7.54 (d, 2H, *J*=8.68 Hz, meta protons of *p*-tolyl), 8.25 (d, 2H, *J*=9.0 Hz, meta protons of *p*-nitrophenyl), 8.51 (d, 2H, *J*=9.0 Hz, ortho protons of *p*-nitrophenyl); UPLC-MS (m/z) : 363 (M^++1).

2-[5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-methoxyphenyl)-1,3,4-oxadiazole 3d

IR (KBr): 2964 (C-H), 1610 (C=N), 1526 (asym. NO_2), 1378 (sym. NO_2); ^{1}H NMR (DMSO): 2.60 (s, 3H, triazole CH_3), 3.26 (s, 3H, OCH_3), 7.26 (d, 2H, *J*=8 Hz) meta protons of *p*-methoxyphenyl), 7.86 (d, 2H, *J*= 8Hz, ortho protons of *p*-methoxyphenyl), 7.96 (d, 2H, *J*=9 Hz, meta protons of *p*-nitrophenyl), 8.64 (d, 2H, *J*= 8.9 Hz, ortho protons of *p*-nitrophenyl); LC-MS (m/z): 378 (M^+).

2-[5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-(4-chlorophenyl)-1,3,4-oxadiazole 3e

IR :(KBr): 2954 (C-H), 1535 (C=N), 1538 (asym. NO_2), 1376 (sym. NO_2); ^{1}H NMR (DMSO): 2.77 (s, 3H, CH_3), 7.68 (d, 2H, *J*=8.0 Hz, meta protons of *p*-chlorophenyl), 8.09 (d, 2H, *J*= 8.2 Hz, ortho protons of *p*-chlorophenyl), 8.14 (d, 2H, *J*=9.0 Hz, meta protons of *p*-nitrophenyl), 8.53 (d, 2H, *J*=9.0 Hz, ortho protons of *p*-nitrophenyl); UPLC-MS (m/z): 383 (M^++1).

2-[5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]-5-[2-chloro-4-fluorophenyl]-1,3,4-oxadiazole 3h

IR (KBr): 2956 (C-H), 1598 (C=N), 1546 (asym. NO_2), 1386 (sym. NO_2); ^{1}H NMR (DMSO): 2.64 (s, 3H, CH_3), 7.45 (s, 1H, ArH), 7.65 (d, 1H, *J*=7.12 Hz, ArH), 7.84 (d, 1H, *J*=7.16 Hz, ArH), 7.99 (d, 2H, *J*=8.96 Hz, meta protons of *p*-nitrophenyl), 8.51 (d, 2H, *J*=8.9 Hz, ortho protons of *p*-nitrophenyl); UPLC-MS (m/z): 401 (M^++1).

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References

1. A.A. El-Emam, O.A. Al-Deeb, M. Al-Omar and J. Lehmann, *Bioorg. Med. Chem.,* **12** (2004), 5107.
2. M. Akhter, A. Husain, B. Azad and M. Ajmal, *Eur. J. Med. Chem.,* **44** (2009), 2372.
3. P.C. Unangast, G.P. Shrum, D.T. Conner, C.D. Dyer and D.J. Schrier, *J. Med. Chem.,* **35** (1992), 3691.
4. A.O. Maslat, M. Abussaud, H. Tashtoush and M. Al-Talib, *Pharmacology*, **54** (2002), 55.

5. A.A. Farghaly, A.A. Bekhit and J.Y. Park, *Arch. Pharm. (Weinheim),* **333** (2000), 53.

6. E.A. Sheremet, R.I. Tomanov, E.V. Trukhin and V.M. Berestovitsakaya, *Russ. J. Org. Chem.,* **40** (2004), 594.

7. L. Savini, P. Massrelli, L. Chiasserini, C. Pellerano and G. Bruni, *Farmaco,* **49** (1994), 633.

8. Nithinchandra, B. Kalluraya, S. Aamir and A.R. Shabaraya, *Eur. J. Med. Chem.,* **43** (2012), 1715.

9. B. Kalluraya, A. Rahiman and B. David, *Arch. Pharm. Med. Chem.,* **334** (2001), 263.

10. K.V. Sujith, J.N. Rao, P. Shetty and B. Kalluraya, *Eur. J. Med. Chem.,* **44** (2009), 3697.

11. J.H. Goh, H.K. Fun, Nithinchandra and B. Kalluraya, *Acta Cryst.,* **E66** (2010), o1303.

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