

Original Article

Synthesis, characterization and biological activities of compounds containing five membered heterocyclic ring system

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ABSTRACT

Oxadiazoles are five membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. Oxadiazoles are synthesized by ring condensation and rearrangement reactions. In this work oxadiazoles are synthesized from naphthoxy acetic acid. The newly synthesized substituted 1,3,4-oxadiazoles were characterized by IR, NMR and Mass Spectrometry. The biological evaluation of compound was carried by determining the minimum inhibitory concentration (MIC) of the test substances against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* by liquid broth method of two fold serial dilution technique. The oxadiazole derivatives were also screened for their anticonvulsant activity by pentylenetetrazole (scPTZ) method. Anti bacterial activity results revealed that compounds IIIa, IIIc, IIIg, IIIh, IIIi, IIIj showed more than 80% inhibition against *V cholerae*, *Shdysenteriae*, *Escherichia coli* and *Klebsiella pneumoniae* bacterial strains. Synthesised compounds also exhibited anticonvulsant activity in scPTZ seizure model.

1. INTRODUCTION

Oxadiazole is important heterocyclic ring present in a large number of biological active molecules of different pharmacological classes. 1,3,4-oxadiazole is a liquid, which boils at 150°C [1]. Ainsworth first prepared it in 1965 by the thermolysis of ethylformateformlyhydrazone at atmospheric pressure. 1,3,4-oxadiazole is a thermally stable aromatic molecule other aromatic system are 1,3,4-oxadiazoliumcation and the exocyclic-conjugated meso ionic-1,3,4-oxadiazole and 1,3,4-oxadiazolines [2-4]. Also known as derivatives of the non-aromatic reduced system, 2,3 dihydro-1,3,4-oxadiazole, 2,5-dihydro-1,3,4-oxadiazole and 2,3,4,5-tetrahydro-1,3,4-oxadiazole [5]. It is known to have fungicidal, bacterial and herbicidal activities. The synthesis of novel oxadiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades for biological, medical and agricultural reasons. Different classes of oxadiazole compounds possess an extensive spectrum of pharmacological activities. In particular, compounds bearing 1,3,4-oxadiazole nucleus are known to exhibit unique anti-edema

and anti-inflammatory activity [6-7]. Differently substituted oxadiazole moiety has also been found to have other important activities such as analgesic antimicrobial, antimycobacterial, anticonvulsant, antitumor, antimalarial and anti-hepatitis B viral activities. Substituted 1,3,4-oxadiazoles exhibit antibacterial, pesticidal and antifungal activities. 1,3,4-oxadiazoles are biologically active, synthetically useful and important heterocyclic compounds. For these reasons the chemistry of 1,3,4-oxadiazoles have been the subject of many investigations [8-9]. One pot synthesis of 1,3,4-oxadiazoles by the reaction of appropriate hydrazide and carboxylic acid has been reported. Ferric ammonium nitrate has received considerable attention as an inexpensive and easily available catalyst for various organic reactions such as Oxidation, Oxidative addition, Nitration, Photo-oxidation, Polymerization etc [10].

2. MATERIALS AND METHOD

All chemicals and solvents were purchased from Qualigens and were of AR-grade purity. All reactions are carried out at laboratory condition. Melting points were determined with

open capillary MP Apparatus. FT-IR spectra were recorded on a Shimadzu FT-IR model 2000 spectrophotometer. ¹H-NMR spectra were recorded on a Varian Mercury FT-NMR model YH-300 instrument using TMS as internal standard. Mass spectra were recorded on GC-MS auto tune EI instrument. The commercially available grades of solvents and reagents were found to be of adequate purity. However, the presence of undesirable impurities and others were likely to be used for experimental work was purified/dried. For TLC, the silica gel G (160-120 mesh) used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Two solvent system were used Benzene: Acetone (9:1) and (8:2), Toluene: Ethyl Acetate: Formic acid (5:4:1). Ashless Whatmann No.1 filter paper was used for vacuum filtration. The investigations were conducted on albino mice of either sex (25-30g). The albino mice were kept under standard condition at an ambient temperature of 25±2°C. Food and water were withdrawn prior to the experiments.

3. EXPERIMENTAL

Newly synthesized substituted 1,3,4-oxadiazoles were synthesized by the following scheme (Fig. 1).

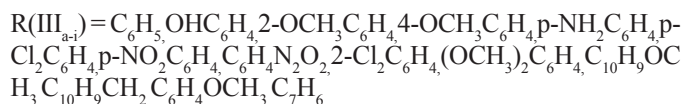
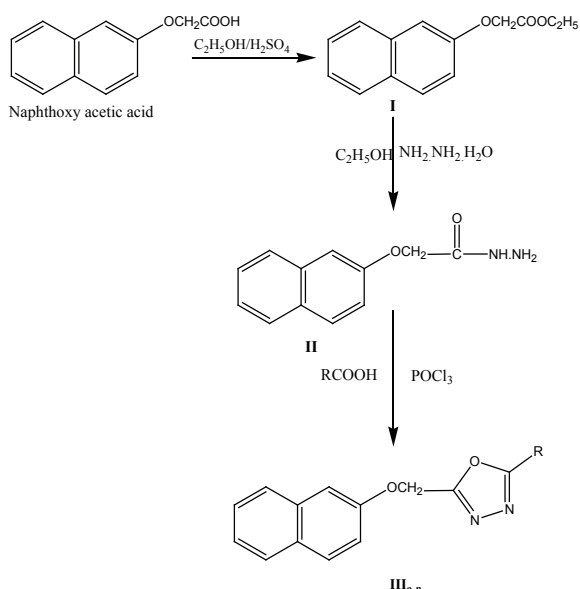


Fig. 1. Synthetic scheme for synthesis of 1,3,4 oxadiazole derivatives

3.1 Synthesis of Naphthalen-2-yloxy-acetic acid ethyl ester (I)

Esterification of Naphthoxy acetic acid was done by reacting it with ethanol in the presence of sulphuric acid resulting in the formation of Naphthalen-2-yloxy-acetic acid ethyl ester (I). (Naphthalen-2-yloxy)-acetic acid ethyl ester(I): Melting point- 69.2-70.8° CM. F- C₁₄H₁₄O₃ M.W-230 IR(KBr)cm⁻¹-1260(C-O); 1665(C=N); 829(N-N); 3192(CH-Ar). ¹H-NMR (DMSO-d₆)δ ppm -4.24, 4.22 (S,2H,CH₂); 4.89(s,1H,OCH₂); 7.11-7.85(m,7H,Aromatic). Mass(m/z)^{-M+}230.

3.2 Synthesis of Naphthalen-2-yloxy-acetic acid hydrazide (II)

Naphthalen-2-yloxy-acetic acid ethyl ester (I) was reacted with hydrazine hydrate in ethanol to form the corresponding hydrazide. (Naphthalen-2-yloxy)-acetic acid hydrazide(II): Melting point-186-187° CM.F- C₁₂H₁₂N₂O₂ M.W-216 IR(KBr) cm⁻¹-1083(N-N); 1258(C-O); 1688(C=O); 3074(CH-Ar); 3025(N-H);1586(C=N).¹H-NMR (DMSO-d₆)δ ppm - 4.88 (s, 2H, OCH₂), 7.19–7.84 (m, 7H, Aromatic); 9.43(s, 1H, CONH).

3.3 Synthesis of 1,3,4 oxadiazole derivatives

A mixture of Naphthalen-2-yloxy-acetic acid hydrazide (II) (0.0025 mole) and derivatives of benzoic acid was refluxed in the presence of POCl₃ (10-15ml) for 6-10 hrs at a temperature of about 110-120° C. After completion of reaction, mixture was cooled at room temperature and poured into crushed ice. On basification of sodium bicarbonate (5%) a solid mass was so separated was washed with water and crystallized from ethanol. On TLC examination in TEF (5:4:1) compound gave single spot. The detailed TLC evaluation is mentioned in Table 1.

3.3.1 2-(Naphthalen-2-yloxymethyl)-5-phenyl-[1,3,4]oxadiazole (III_a): Melting point - 172-174° C.M.F- C₁₉H₁₄N₂O₂ M.W-302 IR(KBr)cm⁻¹-1017(N-H); 1263(C-O); 1627(C=N); 2931(CH-Ar). ¹H-NMR (DMSO-d₆) δ ppm -4.01(s, 2H, OCH₂); 6.87-7.95(m, 12H, Aromatic).

3.3.2 2-(Naphthalen-2-yloxymethyl)-phenol (III_b): Melting point - 170-172° CM.F- C₁₇H₁₄O₂ M.W-1157 IR(KBr)cm⁻¹-1041 (N-N); 1219 (C-O); 1518(C-N); 2919(O-H); 3074(CH-Ar). ¹H-NMR (DMSO-d₆) δ ppm - 5.41 (s, 2H, OCH₂ naphthoxy), 7.09–7.85 (m, 15H, Aromatic), 6.43(s, 1H, OH)

3.3.3 5-Methoxy-2-[5-(naphthalen-2-yloxymethyl)-[1,3,4]oxadiazole-2-yl]phenol (III_c): Melting point - 169-170° CM.F- C₂₀H₁₆N₂O₃ M.W- 332 IR(KBr)cm⁻¹- 1126 (N-N); 1298 (C-O); 1500 (C-N). ¹H-NMR (DMSO-d₆) δ ppm -2.46 (s, 3H, OCH₃), 4.05 (s, 2H, OCH₂), 7.35–8.93 (m, 11H, aromatic).

3.3.4 2-(4-Methoxy-phenyl)-5-(naphthalen-2-yloxymethyl) [1,3,4]oxadiazole (III_d): Melting point - 102-104° CM.F- C₂₀H₁₆N₂O₃ M.W-332 IR(KBr)cm⁻¹-1089 (N-N); 1223 (C-O); 1500(C-N); 2937(CH-Ar). ¹H-NMR (DMSO-d₆) δ ppm -3.10(s, 2H, OCH₂), 3.86 (s, 2H, OCH₃), 7.40-7.81 (m, 11H, aromatic).

3.3.5 4-[5-(naphthalen-2-yloxymethyl)[1,3,4]oxadiazole-2-yl]-phenyl amine (III_e): Melting point -204-208° CM.F- C₁₉H₁₅N₃O₂ M.W-317 IR(KBr)cm⁻¹-1078 (N-N), 1256 (C-O), 1605 (C=N). ¹H-NMR (DMSO-d₆) δ ppm -4.58 (s, 2H, OCH₂), 5.64 (s, 2H, OCH₂), 7.15–7.89 (m, 11H, aromatic); 2.44(s,1H,NH₂).

3.3.6 2-(2-Choloro-phenyl)-5-[naphthalen-2-yloxymethyl] [1,3,4]oxadiazole (III_f): Melting point - 108-110° CM.F- C₁₉H₁₃ClN₂O₂ IR(KBr)cm⁻¹- 828(C-Cl); 1219 (C-O); 1591 (C=N); 3266 (CH-Ar). ¹H-NMR (DMSO-d₆) δ ppm -4.73 (s, 2H, OCH₂), 7.25-7.96 (m, 11H, aromatic).

3.3.7 2-[naphthalen-2-yloxymethyl]-5-(2-nitro-phenyl)[1,3,4]oxadiazole (III_g): Melting point - 80-84° CM.F- C₁₉H₁₃N₃O₄ M.W-347 IR(KBr)cm⁻¹-1031 (N-N); 1277 (C-O); 1513 (NO₂); 1604 (C=C); 3305 (CH-Ar). ¹H-NMR (DMSO-d₆) δ ppm -4.56 (s, 2H, OCH₂), 7.35-7.97 (m, 11H, aromatic).

3.3.8 2-(3,5-Dinitro-phenyl)-5(naphthalen-2-yloxymethyl)-[1,3,4]oxadiazole (III_n): Melting point - 155-157° CM.F- C₁₉H₁₂N₄O₆. M.W-392 IR(KBr)cm⁻¹- 1030(N–N); 1276 (C–O); 1757 and 1604 (NO₂); 1514 (C=N); 3239(CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm – 4.59 (s, 2H, OCH₂), 7.73-8.06 (m, 10H, aromatic).

3.3.9 2-(2-Chloro-phenyl)-5(naphthalen-2-yloxymethyl)-[1,3,4] oxadiazole (III_i): Melting point - 108-110° CM.F- C₁₉H₁₃ClN₂O₂. M.W-336 IR(KBr)cm⁻¹- 784 (C–Cl); 1032 (N–N), 1277 (C–O), 1603 (C,N), 3301(CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm –4.73 (s, 2H, CH₂), 6.17 (s, 2H,OCH₂), 7.73-8.06 (m, 10H, aromatic).

3.3.10 2-(3,5-Dimethoxy-phenyl)-5-(naphthalen-2-yloxy-methyl)-[1,3,4]oxadiazole (III_j): Melting point -160-164° CM.F- C₂₁H₁₈N₂O₄. M.W-362 IR(KBr)cm⁻¹-1079(N–N), 1220 (C–O), 1623 (C,N), 3055 (CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm -4.61 (s, 2H, OCH₂), 5.12 (s,2H, OCH₃), 5.39 (s, 2H, OCH₂), 6.97–7.67 (m, 10H, aromatic).

3.3.11 2,5-Bis-(naphthalen-2-yloxymethyl)-[1,3,4] oxadiazole (III_k): Melting point - 204-208° CM.F- C₂₄H₁₈N₂O₃. M.W-382 IR(KBr)cm⁻¹-1031 (N–N), 1250 (C–O), 1605 (C,N). ¹H-NMR (DMSO-d₆) δ ppm –5.64 (s, 2H, OCH₂), 7.09–7.76 (m, 14H, aromatic).

3.3.12 2(-2-Naphthalen-2-yl-ethyl)-5-(Naphthalen-2-yloxymethyl)-[1,3,4]oxadiazole (III_l): Melting point - 220-222° CM.F- C₂₅H₂₀N₂O₂. M.W-380 IR(KBr)cm⁻¹- 1037 (N–N), 1246 (C–O), 1598 (C,N), 3067 (CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm –4.0 (s, 2H, NH₂), 4.91 (s, 2H, CH₂), 5.31 (s, 2H, OCH₂), 6.73–7.79 (m, 14H, aromatic).

3.3.13 2-(Naphthalen-2-yloxymethyl)-5-phenoxymethyl-[1,3,4]oxadiazole (III_m): Melting point - 187-190° CM.F- C₂₀H₁₆N₂O₃. M.W-332 IR(KBr)cm⁻¹- 1168 (N–N); 1257 (C–O); 1508 (C,N); 3041 (CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm - 4.05 (s, 2H, OCH₂), 7.35–8.93 (m, 12H, aromatic).

3.3.14 2-(Naphthalen-2-yloxymethyl)-5-phenethyl-[1,3,4] oxadiazole (III_n): Melting point -187-190° CM.F- C₂₁H₁₈N₂O₂. M.W-330 IR(KBr) cm⁻¹- 1139 (N–N); 1234(C–O); 1531 (C,N); 2857 (CH–Ar). ¹H-NMR (DMSO-d₆) δ ppm – 4.50, 3.97 (s, 2H, CH₂), 4.48 (s,2H, OCH₂), 7.16–8.27 (m, 14H, aromatic).

Table 1. Thin layer chromatography and R_f value of the compounds

S. No.	Com- pounds codes	Solvent System	Propor- tion	Rf value
1.	IIIa	Toluene:Ethylacetate: Formic acid	(5:4:1)	0.76
2.	IIIb	Toluene:Ethylacetate: Formic acid	(5:4:1)	0.69
3.	IIIc	Toluene:Ethylacetate: Formic acid	(5:4:1)	0.76
4.	III d	Toluene:Ethylacetate: Formic acid	(5:4:1)	0.82

5.	IIIe	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.93
6.	III f	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.57
7.	III g	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.72
8.	III h	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.62
9.	III i	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.96
10.	III j	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.41
11.	III k	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.96
12.	III l	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.61
13.	III m	Toluene: Ethylacetate: Formic acid	(5:4:1)	0.72
14.	III n	Toluene:Ethylacetate: Formic acid	(5:4:1)	0.67

4. PHARMACOLOGICAL EVALUATION

4.1 Anti-microbial activity

Two fold serial dilution method or turbidimetric assay is used to detect the drug potency, based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of suitable micro-organisms in a fluid medium to which have been added, graded amounts of the test compounds and known concentration of reference material. Series of dilutions were prepared containing the same volume of medium inoculated with the test organism (the inoculums may vary from 10⁶ to 10⁸ cells / ml). Drug solutions were prepared by serial two-fold dilution technique and one tube was left without drug, to serve as positive control. Serial two fold dilutions of the test compounds (Table 2) were prepared from a high concentration of 1000 jg/ml. The same procedure was followed for positive control. The lowest concentration of the fraction which caused apparently a complete inhibition of growth of organisms was taken as MIC (Table 4).

Table 2. Concentration of drugs in petri dishes

S. No.	Drug in Media	Conc. of Drug 1 mg/ml	Conc. of Drug 10 mg/ml	Conc. of Drug 20 mg/ml
1.	0 µg/ml	----	----	----
2.	5 µg/ml	0.15 ml	----	----
3.	25 µg/ml	0.75 ml	----	----
4.	50 µg/ml	----	0.15 ml	----
5.	100 µg/ml	----	0.3 ml	----
6.	200 µg/ml	----	0.6 ml	----
7.	400 µg/ml	----	----	0.6 ml

Table 3. Antimicrobial activities (MIC, µg/ml) of the title compounds

Comp	R	<i>E. coli</i>	<i>V. cholerae</i>	<i>B. subtilis</i>	<i>Klebsellapneumoniae</i>	<i>Shdysentria</i>
III _a	C ₆ H ₅	200	-	25	-	-
III _b	OHC ₆ H ₄	100	100	100	100	100
III _c	2-OCH ₃ C ₆ H ₄	25	200	25	50	50
III _d	4-OCH ₃ C ₆ H ₄	100	-	>200	100	100
III _e	p-NH ₂ C ₆ H ₄	>200	-	100	50	-
III _f	p-Cl ₂ C ₆ H ₄	12.5	50	12.5	25	50
III _g	p-NO ₂ C ₆ H ₄	25	-	50	50	50
III _h	C ₆ H ₄ N ₂ O ₂	100	-	50	-	-
III _i	2-Cl ₂ C ₆ H ₄	25	25	-	50	12.5
III _j	(OCH ₃) ₂ C ₆ H ₄	>200	100	25	200	200
III _k	C ₁₀ H ₉ OCH ₃	100	25	100	-	-
III _l	C ₁₀ H ₉ CH ₂	12.5	>200	50	100	50
III _m	C ₆ H ₄ OCH ₃	50	100	-	50	25
III _n	C ₇ H ₆	100	25	25	100	100
Ciprofloxacin	-	12.5	20	12.5	20	12.5

4.2 Anticonvulsant activity

Albino mice of either sex weighing between 20- 25 g, were used in the present study. Animals were divided into three groups each comprising of 6 animals. One group was used for studying the effect of PTZ, the second group for control and the third group to study effect with reference to the standard. The subcutaneous dose of pentylenetetrazole (85mg/kg) at which 95% of the animals showed convulsive reaction was determined

by a dose-percent effect curve. The synthesized compounds were administered intraperitoneally. At the anticipated time, PTZ was then administered subcutaneously in the posterior midline of mice. The absence of clonic spasm in half or more of the animals in the observed time periods indicates the compounds capacity to terminate the effect of pentylenetetrazole on seizure threshold. The results of test compound (Table 4) were compared with control and standard compounds.

Table 4. Anticonvulsant activity of 1,3,4 Oxadiazoles derivatives.

S. No	R	Dose mg/kg	Convulsion			Death or Recovery
			Onset (Sec)	Nature of Severity	Clonic time (Sec.)	
01	C ₆ H ₅	0.2	43	Jerkey movement	43-62	4/4
02	OHC ₆ H ₄	0.2	44	Jerkey movement	44-72	4/4
03	2-OCH ₃ C ₆ H ₄	0.2	48	Tail Struabs	48-85	2/4
04	4-OCH ₃ C ₆ H ₄	0.2	46	Jerkey movement	46-92	3/4
05	p-NH ₂ C ₆ H ₄	0.2	58	Jerkey movement	58-89	4/4
06	p-Cl ₂ C ₆ H ₄	0.2	52	Jerkey movement	52-75	3/4
07	p-NO ₂ C ₆ H ₄	0.2	43	Jerkey movement	43-72	4/4
08	C ₆ H ₄ N ₂ O ₂	0.2	42	Jerkey movement	42-69	3/4
09	2-Cl ₂ C ₆ H ₄	0.2	41	Tail Struabs	41-71	4/4
10	(OCH ₃) ₂ C ₆ H ₄	0.2	63	Jerkey movement	63-73	2/4
11	C ₁₀ H ₉ OCH ₃	0.2	45	Jerkey movement	45-77	4/4
12	C ₁₀ H ₉ CH ₂	0.2	42	Jerkey movement	42-75	3/4
13	C ₆ H ₄ OCH ₃	0.2	68	Tail Struabs	68-73	3/4
14	C ₇ H ₆	0.2	66	Jerkey movement	66-108	4/4
15	Control + PTZ	5 ml/kg+80	73	Jerkey movement	73-126	4/4
16	Diazepam+PTZ	4 ml/kg+80	49	Tail Struabs	49-60	0/4

5. RESULTS AND DISCUSSION

The synthesized compounds were subjected to antimicrobial and anticonvulsant evaluation. The antibacterial evaluation of compound was carried by determining the minimum inhibitory concentration (MIC) of the test substances against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* by liquid broth method of two fold serial dilution technique. The oxadiazole derivatives were also screened for their anticonvulsant activity by pentylenetetrazole (scPTZ) method. Anti bacterial activity results revealed that compounds III_a, III_c, III_g, III_h, III_i, III_j showed more than 80% inhibition against *V cholerae*, *Shdysenteriae*, *Escherschia coli* and *Klebsiellapneumoniae* bacterial strains. Synthesized compounds also exhibited anticonvulsant activity in scPTZ seizure model.

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