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ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis and Pharmacological Evaluation of Few Novel 1,3,4-Oxadiazole Derivatives

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ABSTRACT
Multistep reaction sequences were used to synthesize new 1,3,4-oxadiazole compounds. IR, NMR, and mass spectrum analyses were used to characterise and confirm the structure of the synthesized substances. All the newly synthesized compounds were tested for acute toxicity, anti-convulsant and gross behavioural studies. Synthesized compounds were found to have a lot of biological activity, thus they have a lot of potential for finding safer biologically active molecules.

Keywords: *1,3,4-oxadiazole, synthesis, Acute Toxicity, Locomotor Activity, anti-oxidant activity***.**

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INTRODUCTION

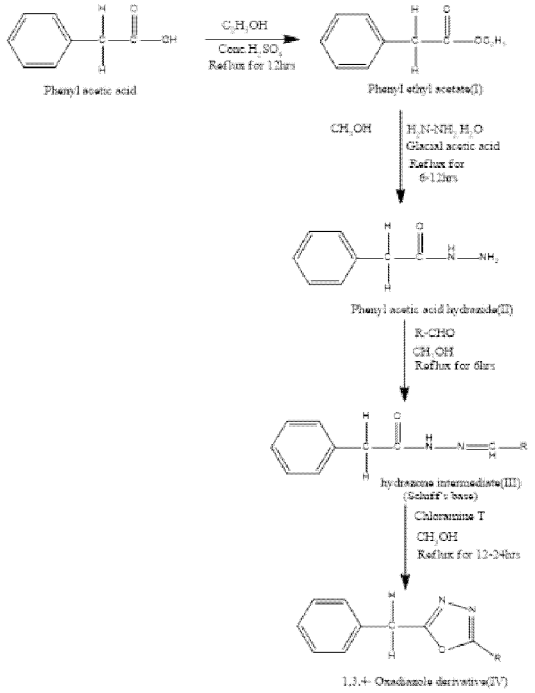
1,3,4-oxadiazoles are a class of heterocyclic compounds with a wide range of biological activity, including antibiotics, analgesics, ulcerogenicity, apoptosis inducers, antimycobacterial, antifungal, anticancer, P-glycoprotein inhibitors, insecticides, and inhibitors of 4-hydroxylase [1]. Oxadiazole is thought to be the outcome of replacing two methane (–CH=) groups with two pyridine type nitrogen atoms (–N=) in furan [2]. For the synthesis of 1,3,4-oxadiazoles, several techniques have been documented in the literature. Acid hydrazides (or hydrazine) reactions with acid chlorides/carboxylic acids and direct cyclization of diacylhydrazines using a variety of dehydrating agents such as phosphorous oxychloride [3], thionyl chloride [4], phosphorous pentaoxide [5], triflic anhydride [6], polyphosphoric acid [7] are the most common synthetic routes for 1,3,4-oxadiazole.
Researchers in the disciplines of medical and pharmaceutical chemistry are interested in heterocyclic molecules containing nitrogen atoms, such as oxadiazole moieties. We investigated the Acute toxicity, Gross Behavioural, Locomotor activity, Anticonvulsant activity, and Antioxidant activity of substituted 1,3,4- oxadiazole derivatives as antibacterial agents in this work.

MATERIAL AND METHODS

Preparation of Phenyethylacteate (I):
For 12 hours, a combination of 0.01mole phenyl acetic acid and absolute ethanol (5 moles) was refluxed with a few drops of strong sulfuric acid. Small amounts of sodium carbonate or sodium bicarbonate powder were added to the mixture until the carbon dioxide effervescence stopped.
Preparation of Phenylaceticacid hydrazide (II):
For 6-12 hours, a combination of phenylethylacetate (1 mole) and Hydrazine hydrate (4 moles) was refluxed with 1 mL glacial acetic acid and ethanol. The reaction mixture was immediately dumped into ice cold water, and the result was filtered out.
Preparation of Hydrazone intermediate (III):
In the presence of abundant glacial acetic acid and ethanol, equimolar amounts of phenyl acetic acid hydrazide (0.001mole) and different aldehyde derivatives (aromatic or aliphatic) (0.001mole) were refluxed for 12 to 24 hours. The reaction mixture was immediately dumped into ice cold water, and the result was filtered out.

Preparation of 1,3,4-Oxadiazole derivatives(IV):

Under reflux for 6 hours, an equimolar combination of hydrazone intermediate (0.1mole) and chloramine T (0.1mole) was left to react in the presence of a few drops of glacial acetic acid and ethanol. The reaction mixture was immediately placed into ice cold water and filtered away. The detailed synthesis route of the 1,3,4-Oxadiazole derivatives is shown in Scheme 1.



R=$C_6H_4NO_2$, C_6H_4OH , $C_6H_3(OCH_3)_2$,$C_6H_4N(CH_3)_2$,C_2H_2Ph,C_3H_6CHO

Scheme 1

Acute Toxicity Study

In this study, healthy and adult male albino swiss mice weighing between 20 and 25 grammes were employed. The animals were separated into six groups of six animals each and fasted for the night. The test chemicals were given intravenously in doses up to 1000 mg/kg body weight, suspended in 0.1 percent sodium CMC. The animals in the control group were just given a vehicle (0.1 percent sodium CMC). The animals were tracked for a month after they were given the test substances to see how many died [11].

Gross Behavioural studies

In this study, healthy albino swiss mice weighing between 20 and 25 g were employed. The animals were fasted for the night and placed into six groups. I.p. dosages of up to 100mg/kg body weight of the test chemicals suspended in sodium CMC were given. The animals in the control group were just given a vehicle (sodium CMC) [12]

Locomotor Activity

The locomotor activity was measured using an actophotometer, which uses photoelectric cells in a circuit with a counter to measure it. A count was kept when animals shut off a ray of light falling on the photocells. Male mice weighing 20-25 grammes were used in this study. The animals were fasted for 24 hours and then separated into six groups. The test chemicals were given i.p. at a dose of 100 mg/kg body weight in normal saline suspension. The animals in the control group were just given a vehicle (Normal saline). After 30 minutes of test chemical delivery, the response (counts) was recorded. The animals were placed in an actophotometer for 10 minutes and their scores were taken (number of deflections) [13].

Anticonvulsant Activity

Chemically induced convulsions

The anticonvulsant activity was investigated using the chemical convulsion inducer leptazole (Pentylenetetrazole). Male mice weighing 20-25 grammes were fasted overnight and put into six groups of six mice each. The mice were given an intraperitonial injection of leptazole (80 mg/kg). The animals that demonstrate convulsions were chosen for the study. The test chemicals were given i.p. at a dose of 100mg/kg body weight in sodium CMC suspension. The animals in the control group were just given a vehicle (Sodium CMC) [14].

Antioxidant activity of 1,3,4-oxadiazole derivatives

To make a 1mM stock solution, the required amount of ascorbic acid was precisely weighed and diluted in distilled water. From a stock solution of Ascorbic acid, solutions of various concentrations (10nM, 30nM, 100nM, 300nM, 1nM) were generated. The appropriate amount of DPPH was dissolved in 100ml of methanol to make a 0.2mM solution. To prevent DPPH oxidation, the solution was kept out of direct sunlight.A 1mM stock solution was made by dissolving the required amount of test chemicals in methanol. The concentrations of the solutions ranged from 10nM to 1mM. 2.5ml of methanol and 0.5ml of DPPH solution were added to 0.1ml of test sample/ascorbic acid, mixed thoroughly, and absorbance was measured at 517nm against a blank produced in the same way but without the test component [15].

The results were plotted on a graph and IC50 value was calculated.

$$\% \text{ inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

RESULT AND DISCUSSION

Physical and spectral analysis of Synthesis of 1,3,4-oxadiazole derivatives

The physical data of the synthesized 1,3,4-oxadiazole derivatives (IV-a to IV-f) are listed out in Table1. Here the yield of the percentages are from 54 to 67%.

Table 1. physical data of the synthesized 1,3,4-oxadiazole derivatives (IV-a to IV-f).

S.No	Compound	R	Molecular formula	Molecular weight	% yield
1	IV-a	$-C_6H_4NO_2$	$C_{15}H_{11}N_3O_3$	282.16	54
2	IV-b	$-C_6H_4OH$	$C_{15}H_{12}N_2O_2$	252.09	63
3	IV-c	$-C_6H_3(OCH_3)_2$	$C_{17}H_{16}N_2O_3$	296.32	54
4	IV-d	$-C_6H_4N(CH_3)_2$	$C_{17}H_{17}N_3O$	279.33	67
5	IV-e	$-C_2H_2Ph$	$C_{17}H_{14}N_2O$	262.30	56
6	IV-f	$-C_3H_6CHO$	$C_{13}H_{14}N_2O_2$	230.26	58

Spectral analysis

Spectral data of 2-methylphenyl-5-nitrophenyl-1,3,4-oxadiazole(IVa):

IR (KBr) cm-1 : 3102.16 (Ar C-H), 1677.58(C=N) , 1474.66(NO2) 1325.61(C-N), 1135.69(C-O), $_1$H NMR (300 MHz) (DMSO)δppm: δ 3.81 (2H,-CH2), δ 7.23(2H,Ar-H), δ 7.33 (2H,Ar-H), δ 7.26(1H, Ar-H), δ 8.23 (2H, Ar-H), δ 8.32 (2H,Ar-H) Mass spectrum (ESI) M+1 peak was observed at 283.

Spectral data of 2-methylphenyl-5-hydroxyphenyl-1,3,4-oxadiazole(IVb):

IR (KBr) cm^{-1} : 3292.45(OH),3091.39(C-H Ar)1681.39(C=N), 1292.25 (C-O), ^{1}H NMR (300 MHz) (DMSO)δppm: δ3.81 (2H,-CH_2), δ 5.35 (1H, OH), δ 7.01 (1H,Ar-H), δ 7.02 (1H,Ar-H), δ 7.07 (1H,Ar-H) , δ 7.24 (1H,Ar-H),δ 7.23(2H,Ar-H), δ 7.33 (2H,Ar-H), δ 7.26(1H, Ar-H) Mass spectrum (ESI) M+1 peak was observed at 253.

Acute Toxicity study

Table 2 lists the acute toxicity results of the newly synthesised 1,3,4-Oxadiazole derivatives..All of the newly synthesised 1,3,4-Oxadiazole derivatives were tested for acute toxicity and were found to be free of toxicity and toxic symptoms even at high doses of 1000 mg/kg (b.w.) intraperitoneally. However, irritation-like behaviour was observed at 1000 mg/kg (b.w.), so the dose was reduced to ten times the dose where the irritability was noticed.

Table 2. Acute Toxicity analysis of the synthesized 1,3,4-Oxadiazole derivatives.

DOSE mg/kg (b.w)	Toxic Symptoms & Irritability
10	-ve
30	-ve
100	-ve
300	-ve
1000	Found Irritability

Gross Behavioural studies

The compound (IV-a) was watched for gross behavioural changes for 3 hours after administration of the test compound and for 48 hours intermittently, and the results were compared to those of the control group of mice. The compound (IV-a) showed CNS depressing action in the gross behavioural testing. At a dose of 100mg/kg, no mortality was seen with the test chemical (b.w). According to preliminary toxicity tests, the test compounds show an excellent safety profile up to and including the highest dose (2500 mg/kg). After 24 hours, no mortality or behavioural changes in the animals were observed in any of the compounds at concentrations of 1000 and 1500 mg/kg, and behavioural alterations were also recorded

for the same dose. The test chemicals' gross behavioural investigations demonstrated that all of them caused CNS depression in mice.

Locomotor activity

Figure 1 shows the percent potency of the synthesized compounds, as well as the mean convulsive threshold. The compounds were put to the test at a 100mg/kg dose (b.w) The data is presented as a mean with a sample size of six. When compared to conventional fluoxetine, compounds IVa, IVb, and IVc significantly reduced locomotor activity, whereas compounds IVd) and IVe (*p 0.05) did not exhibit a significant reduction in locomotor activity. Another interesting observation is that compounds with nitro groups (electron withdrawing groups) at both positions 2 and 5 of the 1,3,4-oxadiazole ring (IVa) have more antidepressant efficacy than the others in the series. All of the compounds that were evaluated for neurotoxicity passed the test, indicating that they did not cause neurological damage. The actophotometer was used to measure locomotor activity. After 30 minutes of test drug delivery, the response (Counts) was recorded. When compared to the control, all of the drugs showed a decrease in locomotor activity. Compounds IVa (R= $-C_6H_4NO_2$); IVc (R= $C_6H_3(OCH_3)_2$) ;IVe (R= C_2H_2Ph)., IVf (R= $-C_3H_6CHO$) showed more reduction in the locomotor activity. Compound IVf(R= $-C_3H_6CHO$) showed good percent decrease in locomotor activity with 64.02% among all the test compounds, whereas 74.9 % decrease was observed with the standard drug phenytoin.

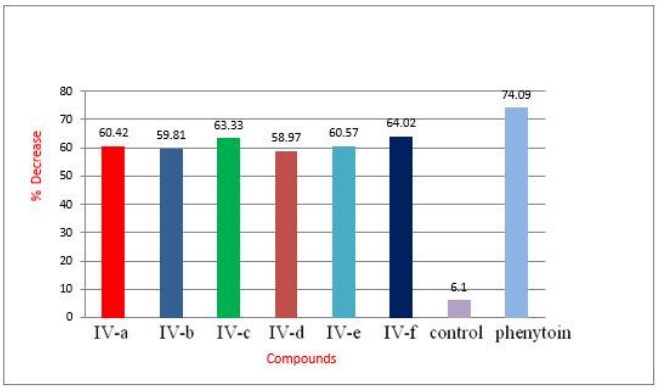


Figure 1. Locomotor activity of 1,3,4-Oxadiazole derivatives

Anticonvulsant Activity

The animals were divided into three groups of six animals each (standard, control, and test) and fasted for 24 hours before the experiment with free access to water. Only 1% (w/v) carboxymethyl cellulose suspension was given to the control group. Phenytoin, a common medication, was given orally at a dose of 30 mg kg-1. The test compounds were given orally at a dose that was equimolar to 30 mg kg-1 phenytoin. Orally, the test chemicals and standard medication were suspended in carboxymethyl cellulose in water (1 percent w/v).The compounds (IVa-IVf) were tested for anticonvulsant activity in vivo using the leptazole induced convulsion method at a dose of 100 mg/kg, with phenytoin as a control.After 30 minutes of treatment, the anticonvulsant activity was evaluated. Table 3 summarises the results of anticonvulsant action.

All the test compounds exhibited anticonvulsant activity in the range of 71.7 to 78% protection. Compound IVc (R= C_6H_3 $(OCH_3)_2$) showed comparatively more activity against Leptazole induced convulsions with 78% inhibition whereas the standard drug Phenytoin produced 88.76 % inhibition. Compound IV-f (R=$-C_3H_6CHO$) , IV-e (R=C_2H_2Ph) , IV-a (R=$-C_6H_4NO_2$) ,IV-b (R=C_6H_4OH), IV-d (R =$-C_6H_4N(CH_3)_2$) showed 76.45%, 74.64%,73.32%, 72.48%, 71.7 % inhibition respectively.

Table 3. Anticonvulsant activity of synthesized compounds

S No	Compound	Animal Protected in %
		Chemically induced convulsion
1.	IV-a	73.32± 1.23
2.	IV-b	72.48± 0.60
3.	IV-c	78.69± 1.321
4.	IV-d	71.7± 0.782
5.	IV-e	74.64± 1.553
6.	IV-f	76.45± 0.975
7.	Phenytoin	88.76± 1.180
8.	Control	0

Antioxidant activity

Among all the compounds, compound IV-c (R= -$C_6H_3(OCH_3)_2$) showed good free radical scavenging activity with IC_{50} value of 62.82µM . Compounds IV-a (R= -$C_6H_4NO_2$) , IV-b (R=- C_6H_4OH) were found in the next order of free radical scavenging activity with IC_{50} values 64.41µM, 64.31µM respectively. Table 4 shows Antioxidant activity of 1,3,4-oxadiazole derivatives of all the compounds shown in Table 4.

Table 4. Antioxidant activity of 1,3,4-oxadiazole derivatives

S No	Compound	Animal Protected in %
		Chemically induced convulsion
1.	IV-a	64.41
2.	IV-b	64.31
3.	IV-c	62.82
4.	IV-d	70.32
5.	IV-e	65.75
6.	IV-f	105.42
7.	Ascorbic Acid	16.55

CONCLUSION

This study demonstrated the cyclization of the -NH-NH_2 group of benzohydrazide into 1,3,4-oxadiazole in the presence of the carboxylic group of benzoic acid, with the goal of developing compounds with high yield and short reaction times. All of the test compounds used in the study were determined to be devoid of toxicity and toxic symptoms, and gross behavioural investigations demonstrated that the compounds (IV-a-IVf) had anticonvulsant properties in the test animals. At a dose of 100mg/kg, no mortality was seen with the test substances. The data on locomotor activity of the compounds, revealed that the compound IV-f (R=-C_3H_6CHO) showed more reduction (**64.02%**) in locomotor activity among all the test compounds, when compared with the standard drug phenytoin. The compound IV-c(R= $C_6H_5(OCH_3)_2$showed comparatively more anticonvulsant activity with 78% protection against chemically induced convulsions (Leptazole) whereas standard drug Phenytoin exhibited 88.7% protection.Overall, all compounds could be selected as the most physiologically active members in this investigation, having good anticonvulsant and antidepressant activity based on the overall results.

CONFLICTS OF INTEREST

There are no conflicts of interest

REFERENCES

1. Aydemir N, Bilaloğlu R (2003). Genotoxicity of two anticancer drugs, gemcitabine and topotecan, in mouse bone marrow in vivo. Mutat Res. 537:43-51.
2. Bhardwaj, N., Saraf, S. K., Sharma, P., & Kumar, P. (2009). Syntheses, evaluation and characterization of some 1, 3, 4-oxadiazoles as antimicrobial agents. *E-journal of Chemistry*, *6*(4), 1133-1138.
3. Kadi AA, El-Brollosy NR, Al-Deeb OA, Habib EE, Ibrahim TM, El-Emam AA. (2007). Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1, 3, 4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1, 3, 4-thiadiazoles. European journal of medicinal chemistry.;42(2):235-42.
4. Mickevičius V, Vaickelionienė R, Sapijanskaitė B. (2009). Synthesis of substituted 1, 3, 4-oxadiazole derivatives. Chemistry of heterocyclic compounds. ;45(2).20-26
5. Bentiss F, Lagrenee M. (1999). A new synthesis of symmetrical 2, 5-disubstituted 1, 3, 4-oxadiazoles. Journal of heterocyclic chemistry. ;36(4):1029-32.
6. Liras S, Allen MP, Segelstein BE.(2000). A mild method for the preparation of 1, 3, 4-oxadiazoles: Triflic anhydride promoted cyclization of diacylhydrazines. Synthetic Communications.;30(3):437-43.
7. Iosip MD, Bruma M, Robison J, Kaminorz Y, Schulz B. (2001). Study of related poly (1, 3, 4-oxadiazole-amide) s containing silicon or hexafluoroisopropylidene groups in the main chain. High Performance Polymers.;13(3):133-48.
8. Roy PP, Bajaj S, Maity TK, Singh J. (2017). Synthesis and evaluation of anticancer activity of 1, 3, 4-oxadiazole derivatives against Ehrlich ascites carcinoma bearing mice and their correlation with histopathology of liver. Indian J Pharm Educ. 15:16.
9. Eckhardt S. (2002). Recent progress in the development of anticancer agents. Current medicinal chemistry-anti-cancer agents. ;2(3):419-39.
10. Datta S, Saha P, Dey S, Sinha D. (2020). Natural Products as Chemosensitizers for Adjunct Therapy in Cancer Management. In Pharmacotherapeutic Botanicals for Cancer Chemoprevention(pp. 67-119). Springer, Singapore.
11. Halim SZ, Abdullah NR, Afzan A, Rashid BA, Jantan I, Ismail Z. (2011). Acute toxicity study of *Carica papaya* leaf extract in Sprague Dawley rats. Journal of Medicinal Plants Research. ;5(10):1867-72.

12. Emck C, Bosscher R, Beek P, Doreleijers T. (2009). Gross motor performance and self-perceived motor competence in children with emotional, behavioural, and pervasive developmental disorders: a review. Developmental medicine & child neurology. ;51(7):501-17.
13. Dietz V, Colombo G, Jensen L. (1994). Locomotor activity in spinal man. The lancet. ;344(8932):1260-3.
14. Verma M, Pandeya SN, Singh KN, Stables JP. (2004). Anticonvulsant activity of Schiff bases of isatin derivatives. ActaPharmaceutica. ;54(1):49-56.
15. Ma L, Xiao Y, Li C, Xie ZL, Li DD, Wang YT, Ma HT, Zhu HL, Wang MH, Ye YH. (2013). Synthesis and antioxidant activity of novel Mannich base of 1, 3, 4-oxadiazole derivatives possessing 1, 4-benzodioxan. Bioorganic & medicinal chemistry. ;21(21):6763-70.

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