Research Article

Microwave Assisited Synthesis of Some Substituted Heterocycles and Evaluation of their Anticonvulasant Activity

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ABSTARCT

Few 2,4–imidazolidinedione derivatives containing substituted 1,3,4-oxadizoles were synthesized and evaluated for anticonvulsant activity using Pentylenetetrazole (PTZ) induced convulsions in mice. Scientific Microwave Synthesizer was used as heating source for carrying out the reactions. For all the compounds, the yield obtained was in the range of 80 to 95%. The compounds were characterized by IR, ^{1}H NMR and MS. Compounds B1211, C0811 and G1211 showed maximum protection against PTZ induced convulsions in mice in the test group at the dose of 15mg/Kg. Diazepam (2mg/kg) was used as standard for the evaluation of anticonvulsant activity.

INTRODUCTION[1-4]

Hydantoins are the most active class of compounds having anticonvulsant activity. 1,3,4-oxadiazoles represent one of the most active class of compounds possessing wide spectrum of biological activities including anticonvulsant activity. There are several reports on synthesis of substituted hydantoins and that of oxadiazoles using microwave technology but there is need to develop the molecules bearing both the heterocycles in their structure with the expectation to have more efficacious anticonvulsants with a potential to be carried forward through preclinical, toxicokinetic, pharmacokinetic and clinical evaluation. Based on our previous research on optimization of microwave assisted reaction conditions like microwave power, reaction time and quantity of catalyst etc. using systematic statistical designs, few derivatives of hydantoins containing substituted 1,3,4oxadizoles were synthesized by applying the optimized microwave assisted reaction conditions. The compound obtained were purified, characterized and evaluated for anticonvulsant activity using animal models.

EXPERIMENTAL[5-8]

All the chemicals viz. various 5,5-disubstituted 2,4-imidazolidinediones were synthesized by reported methods. Ethyl Chloroacetate, Hydrazine Hydrate (99%), various aromatic aldehydes and solvents were purchased from Sigma-Aldrich Co. Microwave assisted synthesis was carried out using Scientific Microwave Synthesizer from Catalysts Systems (CATA 2R), ranging from power levels 1 (140 watts) to High (700 Watts). Melting points were taken in open glass capillary using Elico melting point apparatus and were uncorrected. Thin-layer chromatography was done with silica gel G as adsorbent. The spots were detected by exposure to iodine vapors. Infra-Red spectra of compounds were recorded on Shimadzu I.R. Affinity-1 FT-IR Spectrophotometer model using Pellet technique. Proton (^{1}H) Nuclear Magnetic Resonance spectra of compounds were recorded on Bruker Avance II 400 NMR Spectrophotometer using $CDCl_3$ solvent, at SAIF, Punjab University, Chandigarh. Mass spectra of compounds were recorded on API 4000 Q TRAP LC/MS/MS system using electron spray ionization positive ion mass spectrometric technique, at NHRDF, Chitegaon, Nashik. The following solvent

systems were used for Thin Layer Chromatography.
A:Benzene: Methanol::4:1
B: Ethyl acetate: Acetone:: 4:1

General procedure for Synthesis of 3-[(5'-substituted 1,2,4-oxadizole-2-yl)-methyl]-5,5-disubstituted-2, 4-imidazolidinediones: (Scheme 1)
A mixture of equimolar quantities of 5,5-disubstituted-2, 4-imidazolidinedione-3-yl acetic acid hydrazide, substituted aromatic aldehyde and appropriate quantity of Phosphorous oxychloride ($POCl_3$), in 10 mL dichloromethane was irradiated under microwaves at the power and for the time mentioned in **Table 1** . Reaction mixture was cooled to room temperature, poured into 50 gm of crushed ice and stirred vigorously. Deep freezing was done overnight. Solid thus obtained was filtered, air dried and recrystallized from Ethanol. Purity of the product was checked by TLC.

Evaluation of anticonvulsant activity by Pentylenetetrazole (PTZ) induced convulsions in mice[9-14]
Animals
Swiss Albino mice of either sex, weighing 22-30 gm were used for the study. The animals were purchased from Hafkine's Institute, Parel, Mumbai, India. Animals were housed in different groups consisting of five animals in each group, in plastic cages under good hygienic conditions in registered animal house of MET's Institute of Pharmacy, Nashik (Registration no. 1344/ac/10/CPCSEA). Bedding of rice husk was replaced twice in a week so as to maintain good hygienic conditions. Ambient temperature of 25±1 ºC, relative humidity of 45-55% and 12 hrs light: 12 hrs dark cycles were maintained in the animal house. The animals had free access to water and standard pelleted diet except during experimentation food and water was withheld.
Preparation of doses
The drugs and chemicals were freshly prepared. Pentylenetetrazole (Dose: 80 mg/kg, i.p.), a stock solution containing 8 mg/mL was prepared by dissolving it in distilled water. Diazepam (Dose: 2 mg/kg, i.p.), a stock solution containing 0.2 mg/mL was prepared by dissolving it in distilled water. All the test compounds were insoluble in water hence they were dissolved in DMSO. The doses of test compounds were 10 mg/kg, i.p. and 15 mg/kg, i.p. respectively and stock solutions of the test compounds containing 1 mg/mL and 1.5 mg/ mL were prepared by dissolving the test compounds in DMSO. The injection volume was 1mL/100 gm of body weight of animal.

Procedure
1. The animals were first weighed and were selected for the experiment depending on the weight. The animals were then divided into sixteen groups, of five animals each. One group is used for studying the effects of Pentylenetetrazole alone (Control) and the other for studying the protective effects of Diazepam (Standard). The remaining fourteen groups were used for studying the effects of synthesized compounds (Test). Two doses were used for administration of test compounds, 10 mg and 15 mg/kg i.p.
2. Pentylenetetrazole (80 mg/kg, body weight) was administered intraperitoneally to induce convulsions in the control and the onset of convulsions, severity of convulsions and mortality was noted.
3. PTZ (80 mg/kg, i.p.) was administered half an hour after the administration of Diazepam and the test compounds. In case of Diazepam treated animals, either delay or complete abolition of convulsions was noted. The test group animals were observed for onset of convulsions, number of convulsions and percentage of protection. The Diazepam treated and test animals were observed following PTZ injection up to half an hour.

RESULTS AND DISCUSSION

Synthetic aspects

Compounds containing heterocycles like hydantoins and 1,3,4-oxadizole can be synthesized by using microwave technology after optimizing the reaction parameters like microwave power, irradiation time, quantity of the catalyst using systematic statistical designs. The yield of the compounds obtained is considerably increased by microwave irradiation as compared to conventional heating technology. The microwave reaction parameters and physicochemical properties of the compounds are shown in **Table 1**.

Spectral analysis[15-16]

3-[(5'-(2-furyl)-1,2,4-oxadizole-2-yl)-methyl]- 5,5-diphenyl -2, 4-imidazolidinedione

(A1211). IR (KBr cm^{-1}): 1235 (asymmetric C-O-C), 1038(Symmetric C-O-C), 3055 (Ar. C-H str); ^{1}H NMR ($CDCl_3$, δ ppm) 7.36 (d, $4H_a$, phenyl), 7.48 (dd, $4H_b$, phenyl), 7.58 (dd, $2H_c$, phenyl), 6.60 (s, $1H_d$, N^1-imidazolidinedione), 4.60 (s, $2H_e$, -CH_2-), 6.72(d, $2H_f$, Furyl), 7.89 (d, $1H_g$, Furyl).

3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]-5,5-diphenyl-2,4-imidazolidinedione (B1211). IR (KBr cm^{-1}): 1222 (asymmetric C-O-C), 1018(Symmetric C-O-C), 3099 (Ar. C-H str), 2963 (methyl C-H); ^{1}H NMR ($CDCl_3$, δ ppm) 7.06 (d, 4H, phenyl) 7.14 (dd, 4H, phenyl) 7.07 (dd, 2H, phenyl), 6.0 (s, 1H, N^1-imidazolidinedione) 4.42 (s, 2H, -CH_2-), 7.37 (d, 2H, Phenyl) 6.83 (d, 2H, Phenyl), 3.73(s,3H, OCH_3), MS: (API 4000 Q TRAP LC/MS/MS electron spray ionization) m/z 440 (molecular ion)

3-[(5'-(o-chlorophenyl)-1,2,4-oxadizole-2-yl)-methyl]-5-methyl-5-(p-methoxy phenyl)-2, 4-imidazolidinedione (C0811).

^{1}H NMR ($CDCl_3$, δ ppm) 7.01 (d, 2H, phenyl,) 6.72 (d, 2H, phenyl), 3.73 (s,3H, OCH_3), 6.0 (s, 1H, N^1-imidazolidinedione) 4.42 (s, 2H, -CH_2-), 7.37 (d, 2H, Phenyl) 7.33 (d, 1H, Phenyl), 7.16 (d, 1H, Phenyl), 7.20 (d, 1H, Phenyl), 7.42 (d, 1H, Phenyl),1.92 (s,3H, CH_3-)

3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]-5,5-di (p-methyl phenyl) -2, 4-imidazolidinedione (K1211)

IR (KBr cm^{-1}): 1208 (asymmetric C-O-C), 1058(Symmetric C-O-C), 3076 (Ar. C-H str),2925(methyl C-H) ; ^{1}H NMR ($CDCl_3$, δ ppm) 7.16 (m, 8H, phenyl,) 2.33 (s, 6H, p-CH_3), 6.91 (s, 1H, N^1-imidazolidinedione) 4.60 (s, 2H, -CH_2-), 7.66 (d, 2H, Phenyl), 7.34 (d, 2H, Phenyl),3.83 (s,3H, -OCH_3).

3-[(5'-(p-nitrophenyl)-1,2,4-oxadizole-2-yl)-methyl]- 5,5-di(p-methylphenyl) -2, 4-imidazolidinedione (R1211)

^{1}H NMR ($CDCl_3$, δ ppm) 7.04 (m, 8H, phenyl,) 2.33 (s, 6H, p-CH_3), 4.21 (s, 2H, -CH_2-), 7.35 (d, 2H, Phenyl), 8.39 (d, 2H, Phenyl).

3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]-5-methyl-5-(p-chlorophenyl)-2,4-imidazolidinedione (V1211)

^{1}H NMR ($CDCl_3$, δ ppm) 7.06 (d, 2H, phenyl,) 7.26 (d, 2H, phenyl), 6.06 (s, 1H, N^1-imidazolidinedione) 4.30 (s, 2H, -CH_2-), 7.46 (d, 2H, Phenyl), 6.92 (d, 2H, Phenyl), 3.76 (s,3H,-OCH_3), 1.88(s, 3H, -CH_3).

Pharmacological evaluation

OECD guidelines (No. 425) were followed for acute toxicity studies. Acute oral toxicity in mice was carried out for determining median Lethal Dose (LD_{50}). Animals were dosed two at a time at a minimum of 48 hours intervals. Doses were selected from the sequence 2000, 550, 175, 55, 17.5, 5.5, 1.75 mg/kg with 5 animals per group. Each animal was observed carefully for the signs of toxicity as well as for mortality in the first 30 minutes after dosing and then occasionally for further 4 hours and daily thereafter for a period of 14 days. The number of mice dying during 48 hours period was recorded. Acute intraperitoneal toxicity test was carried out similarly except intraperitoneal route of administration of test compounds was employed. From the observations of toxicity test the dose response graph was plotted and LD_{50} was calculated from the equation of the line obtained. LD50 was

calculated as 100.07 mg/Kg. Thus the two doses 10 mg/Kg (1/10th of LD_{50}) and 15 mg/kg were selected to evaluate anticonvulsant potential of synthesized compound. The anticonvulsant potential of the newly synthesized compounds is evaluated on the basis of the following observations:

- Increase in latency (onset time) to induce convulsions
- Decrease in the number of convulsions
- Percentage protection
-

Formula of percentage protection

$$\% \text{ Protection} = \frac{\text{Number of convulsions of control} - \text{Number of convulsions of test}}{\text{Number of convulsions of control}} \times 100$$

Readings of the test compounds are compared with control.

CONCLUSION

The findings on the current research work suggest the greater anticonvulsant potential in the compounds containing both the heterocycles viz. Hydantoins and 1,3,4-oxadiazoles for the compounds such as B1211, C0811, G1211 which showed maximum latency to induce convulsions as compared to control in mice. Thus 1,3,4-oxadoazole having methoxy- or chloro- substituent on the aromatic ring at its 5-position attached to 5-alkyl/Aryl-5-aryl disubstituted hydantoins through methylene bridge are possible lead for compounds with improved anticonvulsant potential. The other possible spectrum of activity can be evaluated further by using various other animal models for anticonvulsant activity. At the same time Microwave technology serves as a better alternative for conventional heating techniques to improve the yield and purity of the compounds.

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Table 1: Reaction conditions and physicochemical properties of 3-[(5'-substituted 1,3,4-oxadizole-2-yl)-methyl]-5,5-disubstituted-2, 4-imidazolidinediones

Name of the compound	Microwave power (W)	Irradiation time (min.)	Quantity of $POCl_3$ (mL)	% Yield	Melting point (^{0}C)
3-[(5'-(2-furyl)-1,2,4-oxadizole-2-yl)-methyl]-5,5-diphenyl -2, 4-imidazolidinedione **(A1211)**	560	7.5	7.0	92	204-206
3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]- 5,5-diphenyl -2, 4-imidazolidinedione **(B1211)**	455	17	5.5	88	245-248
3-[(5'-(o-chlorophenyl)-1,2,4-oxadizole-2-yl)-methyl]-5-methyl-5-(p-methoxy phenyl)-2, 4-imidazolidinedione **(C0811)**	560	15	1.75	89	234-236
3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]-5-methyl-5-(p-methoxyphenyl)-2, 4-imidazolidinedione **(G1211)**	560	13	1.75	85	262-264

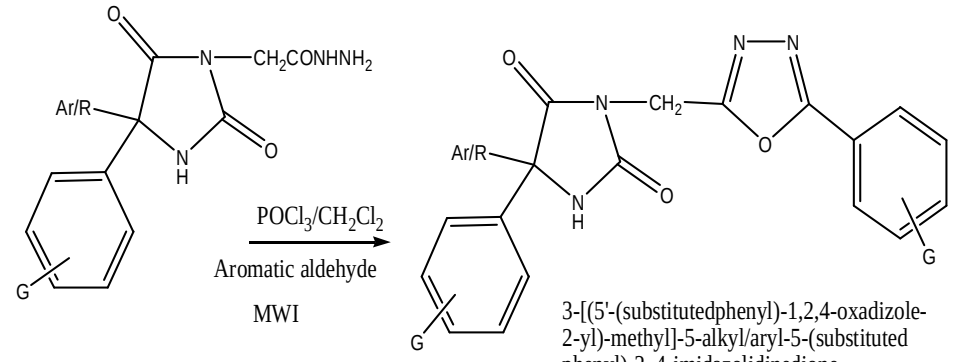
Table 1: (Continued)

Name of the compound	Microwave power (W)	Irradiation time (min.)	Quantity of $POCl_3$ (mL)	% Yield	Melting point (^{0}C)
3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]- 5,5-di(p-methylphenyl) -2, 4-imidazolidinedione **(K1211)**	455	5	3.0	80	244-246
3-[(5'-(p-nitrophenyl)-1,2,4-oxadizole-2-yl)-methyl]- 5,5-di(p-methylphenyl) -2, 4-imidazolidinedione **(R1211)**	490	7	5.0	88	252-254
3-[(5'-(p-methoxyphenyl)-1,2,4-oxadizole-2-yl)-methyl]- 5-methyl-5-(p-chlorophenyl)-2, 4-imidazolidinedione **(V1211)**	455	5	4.5	79	204-206

Table 2: Anticonvulsant effect of 3-[(5'-substituted 1,3,4-oxadizole-2-yl)-methyl]-5,5-disubstituted-2, 4-imidazolidinediones in mice using PTZ induced convulsions method

Compound Code	Dose (mg/Kg, i.p.)	Latency to induce convulsions (Min)	No. of convulsions	% Protection
PTZ (Control)	**80**	1.17±0.33	4	0
Diazepam	**2**	-	0	100
A1211	**10**	3.67±0.31*	3	25
	15	4.51±0.20**	2	50
B1211	**10**	4.14±0.83**	2	50
	15	4.58±0.58**	1	75
C0811	**10**	4.98± 1.09**	2	50
	15	5.25 ±0.25**	1	75
G1211	**10**	3.17±0.52^{NS}	2	50
	15	4.37±0.33**	1	75
K1211	**10**	3.15±0.26^{NS}	3	25
	15	4.79±0.34**	2	50
R1211	**10**	3.67±0.24*	2	50
	15	4.01±0.36*	2	50
V1211	**10**	3.68±0.39*	2	50
	15	4.94±1.33**	1	75

N=5, in each group; *: P < 0.05; **: P <0.01; NS: Non significant;
One Way ANOVA followed by Dunnett's test. Values expressed as Mean ± SEM



5-alkyl/aryl-5- (substitutedphenyl)-2,4-imidazolidinedione-3-yl acetic acid hydrazide

3-[(5'-(substitutedphenyl)-1,2,4-oxadizole-2-yl)-methyl]-5-alkyl/aryl-5-(substituted phenyl)-2, 4-imidazolidinedione

R = $-CH_3$

Ar = $-C_6H_5$, $p\text{-}CH_3C_6H_4$, $p\text{-}OCH_3C_6H_4$

G= -H, $p\text{-}OCH_3$, $p\text{-}CH_3$, o-OH, o-Cl, $p\text{-}NO_2$, p-Cl

Fig. 1: Scheme 1

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