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Microwave irradiated green synthesis of novel isoxazole derivatives as anti-epileptic agent

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Abstract

Green synthetic tools involve the design, synthesis and use of chemical substances by eliminating the generation of chemical hazards. This technique is advantageous in terms of atom economy, use of safer chemicals, energy efficient and decomposition of the waste materials to non-toxic substances which are eco-friendly. So, microwave irradiated heating method is applied for the generation of novel isoxazole derivatives. The structure of isoxazoles moiety contains three carbon atoms, one oxygen atom and one nitrogen atom in the five membered ring. Isoxazole scaffold exhibits diverse pharmacological activities such as anti-viral, anti-tubercular, anti-diabetic, anticancer, anthelmintic, antimicrobial, antifungal, antioxidant, anti-epileptic, antipsychotic etc. A series of novel isoxazole derivatives are synthesized under microwave irradiation. By the help of microwave, the rate of the reaction is enhanced which leads to high selectivity with better product yield as compared to the conventional heating methods. The titled compounds are evaluated for their antiepileptic activity by using maximal electroshock (MES) method. The screening results revealed that some of the compounds exhibited promising antiepileptic activity as compared to the standard drug.

Keywords: Microwave, Green, Synthesis, Epilepsy, Chemistry, Isoxazole, Evaluation

Introduction

Green synthetic protocol involves the utilization of set of principles that eliminates the production of any hazardous substances during the design, manufacture and utilization of chemical substances(1). This method plays a major role to control the pollution of environment by using safer organic solvents, catalysts, suitable reaction conditions there by augments the atom economy and energy efficiency of the synthetic procedure(2). So, the microwave irradiated synthesis of organic compounds is considered a green protocol as it offers numerous benefits over conventional heating techniques(3). The synthesis of organic compounds under microwave irradiation mainly depends on the ability of the reaction medium to absorb microwave energy efficiently and also depends on the selection of organic solvents to complete the chemical synthesis(4,5). Hence, microwave induced synthesis is more advantageous in terms of uniform heating, homogeneity, increased rate of chemical reactions, improvement of product yield and cleaner reaction conditions(6).

Based on this concept, the isoxazole derivatives are synthesized *via* chalcones under microwave irradiated heating method to reduce the by-products formation so that the yield of the final product can be improved in lesser reaction time(7). Isoxazole moiety possess three carbon atoms, one oxygen atom and one nitrogen atom in five-membered ring system(8). Isoxazoles derivatives exhibits diverse biological activities such as antifungal, anti-viral, antimicrobial, anti-tubercular, anti-diabetic, anticancer, antioxidant, anti-epileptic, antipsychotic etc.(9-15).

Isoxazole scaffold forms the basis for different drug molecules such as leflunomide,

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valdecoxib, zonisamide. Similarly, isoxazole derivatives such as sulfamethoxazole, sulfisoxazole and oxacillin are used clinically to treat a wide variety of bacterial infections. (Figure 2) (16-18).

Similarly, chalcone possess α,β-unsaturated carbonyl system that makes these molecules more pharmacologically active (Figure 3) (19-21).

Experimental Section

All the chemicals and reagents used to conduct experiments were of synthetic grade with high purity. The scientific microwave oven was used for the synthesis of isoxazole derivatives. The synthesis of the titled compounds was performed at power level-2 which corresponds to 210W [22-24]. The melting points of the synthesized compounds were determined in open capillary tubes and were observed to be uncorrected. TLC was monitored to check the completion of reaction condition. For TLC study, the solvents such chloroform and ethyl acetate (60: 40) was selected as mobile phase. The sample spots were visualized under ultraviolet lamp. IR spectra of selected compounds was recorded with the help of FT-IR spectrometer (SHIMADZU) by using KBr. Similarly, the ^{1}HNMR spectra was recorded on ^{1}H-NMR spectrometer (Brucker 400 MHz) by using DMSO as solvent and TMS as an internal standard. The synthetic route for the title compounds 4a-e was presented (Scheme1).

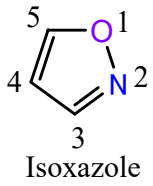

Figure 1. Structure and Nomenclature of Isoxazole Moiety

General Synthesis of Isoxazole Derivatives

Isoxazole derivatives were obtained from chalcone. First of all, chalcone (3a-e) were synthesized by Claisen-Schimdt condensation of 2-hydroxy acetophenone (0.01 mol) and substituted benzaldehydes (0.01 mol) in the presence of sodium hydroxide solution. TLC was monitored to confirm the completion of reaction. Further, the mixture of various chalcones (3a-e) (0.01 mol), hydroxylamine hydrochloride (0.01 mol) in ethanolic sodium hydroxide solution was refluxed under microwave irradiation at 210 W for 10-15 min. TLC was checked to determine the completion of reaction. After completion of the reaction, it was kept on ice bath to obtain solid product of isoxazole derivatives (4a-e).

Antiepileptic Activity

The newly synthesized isoxazole derivatives were evaluated for their

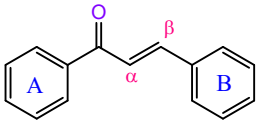

Figure 3. Structure and Nomenclature of Chalcone

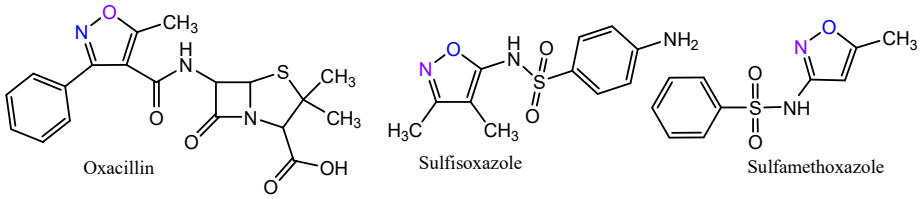

Figure 2. Medicinal Agents containing Isoxazole Pharmacophore

Green Synthesis

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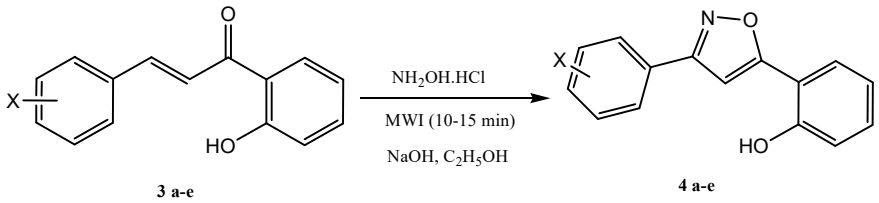

3 a-e 4 a-e

Scheme 1. Synthesis of isoxazole derivatives

Table 1. Antiepileptic Activity of Title Compounds 4(a-e)

Group	Treatment Group	Dose (mg/kg)	HLTE phase duration (sec)	Percentage Protection	Recovery/ Death
Group-I	Control	-	67	-	Death
Group-II	Standard	25	14	79	Recovered
Group-III	4a	300	26	61	Recovered
	4b	300	22	67	Recovered
	4c	300	24	64	Recovered
	4d	300	20	70	Recovered
	4e	300	21	68	Recovered

antiepileptic activity as per the protocols designed by the National Institute of Neurological Disorders and Stroke, NIH (USA). All the animal experiments performed were approved by Institutional Animal Ethics Committee (IAEC), India with protocol no. 926/PO/Re/S/06/ CPCSEA. The antiepileptic activity was performed by using the maximal electroshock (MES) method(25-27). For this study, the rats were selected randomly and each group contains six animals. Group-I was considered as control while Group-II was selected as standard. The remaining groups of animals were administered with all the test compounds with dose of 300 mg/kg. The standard drug (Phenytoin) was injected intraperitoneally 30 min before and the test compounds were administered orally 1h prior to induce the convulsion. Electro convulsive shock (150 mA for 0.2 sec) was given through the corneal electrode to induce convulsions to each group of rats. There are different phases of convulsion which include flexion, extension, clonus and stupor. Prior to delivery, the current output was measured by a multimeter. After the occurrence of electric stimulation, the duration of different phases was determined and the HLTE (Hind limb tonic extension) phase was compared with the control group. A decrease in the duration of hind limb extension was considered as protective action (Table 1, Figure 4). In case of anti-epileptic activity, the percentage protection was calculated as follows.

$$\% \text{ Protection} = \frac{(\text{MEPDnc} - \text{MEPD})}{\text{MEPDnc}} \times 100$$

Where MEPD*nc* is the mean extensor phase duration of normal control in sec., MEPD is the mean extensor phase duration of sample or standard in sec.

Results and Discussion

A new series of isoxazole derivatives (4a-e) was obtained *via* chalcones by

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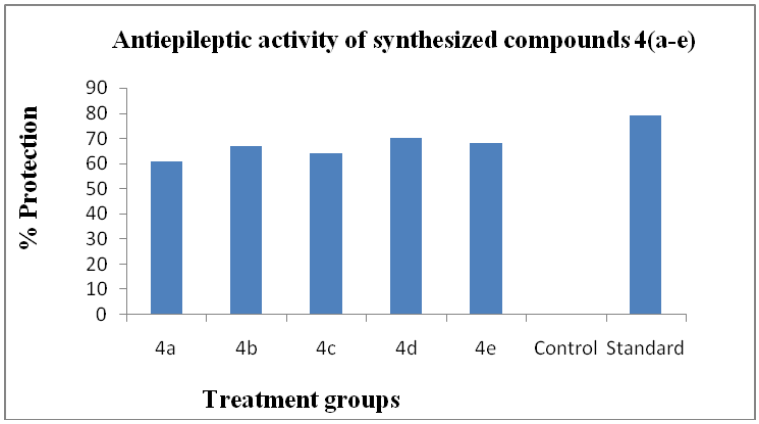

Fig 4. Percentage protection of title compounds

Table 2. Optimization Study of Reaction Time and Product Yield

Comp. Code	X	R_f	M.P (°C)	Conventional Synthesis		Microwave Synthesis	
				Reaction (min)	Yield	Reaction (min)	Yield
4a	H	0.54	148-150	60	64%	10	74%
4b	2-Cl	0.62	150-153	120	56%	12	68%
4c	4-Cl	0.63	155-157	90	63%	13	87%
4d	4-F	0.55	154-159	90	58%	12	65%
4e	4-OH	0.61	162-165	120	60%	10	75%

cyclizaton of chalcones (3a-e) in the presence of hydroxyl amine hydrochloride and ethanolic sodium hydroxide solution. By the help of microwave irradiation, the product yield was improved 65%-87% in short reaction time. The characteristic spectra of α,β unsaturated carbonyl (ketone) group of chalcone was observed near 1640-1650 cm^{-1}. Similarly, the absorption at λ_{max} for various groups are 3220-3300cm^{-1} (-OH), 2924-3116cm^{-1}(Ar-CH), 3029.39 cm^{-1} (aliphatic-C-H), 1630cm^{-1}(C=N), 1735-1750 cm^{-1} (C=O, Ester), 1242-1258cm^{-1}(-C-O-N Str), 1000-1360 cm^{-1}(-C-F), 600-800 cm^{-1} (-C-Cl) respectively. In the ^1H NMR spectrum, the aromatic protons were observed as multiplet at δ6.86-8.56 and singlet at δ6.46 for -OH. The mass spectra of the chalcone and isoxazole derivative exhibited molecular ion peak corresponding to their molecular formula(29-30). The titled compounds were screened for their antiepileptic activity with concentration of 300 mg/ml and the results were compared with standard drug Phenytoin.

Conclusion

Isoxazole derivatives were synthesized by using both the conventional method and microwave irradiation technique to check the product yield. By the help microwave irradiation technique, the product yield was improved from 54% to 87% in a short period of time with

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cleaner reaction conditions. Maximal electroshock method was used for evaluating the antiepileptic activity of the titled compounds The screening results revealed the most of the compounds exhibited promising antiepileptic activity as compared to the standard drug. Further, it is required to focus on optimization study of biological activity in relation to the structural features of isoxazole derivatives to potentiate the development of the therapeutically active compounds.

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