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Microwave-Assisted Facile Synthesis and In vitro Anti-microbial Activities of Some Novel Isoxazole Derivatives *via* Chalcones

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Abstract

A series of some novel isoxazole derivatives were synthesized *via* chalcones under microwave irradiation. Isoxazoles are the unsaturated five-membered heterocyclic aromatic compounds containing three carbon atoms, one oxygen atom, and one nitrogen atom in a ring system. Isoxazole derivatives play a vital role due to their diverse biological activities including antimicrobial, antifungal, anti-viral, anti-tubercular, anti-diabetic, anticancer, anthelmintic, antioxidant, anti-epileptic, antipsychotic, antimalarial, analgesic and anti-inflammatory, etc. Similarly, Chalcones demonstrate diverse pharmacological properties including antioxidant, anti-inflammatory, antiviral, antidiabetic, antispasmodic, and antitumor. These activities are exhibited due to the presence of α, β-unsaturated carbonyl system in chalcone. Hence, chalcones are utilized as a synthon for the synthesis of isoxazole derivatives with diverse structural features. For rapid heating, enhanced rate of reactions, improved product yield, and cleaner reactions, and homogeneity, the microwave-assisted synthetic technique is applied for the synthesis of isoxazole derivatives. The synthesized compounds were characterized by spectral data including FT-IR, ^{1}H-NMR, LC-MS. The titled compounds were evaluated for their anti-microbial activity. Tested compounds exhibited promising anti-microbial activity as compared to standard drugs.

Keywords: Microwave, Synthesis, Isoxazole, Chalcones, spectral data, Anti-microbial.

1. INTRODUCTION

Nitrogen-containing heterocyclic scaffolds are generally considered as major class of compounds in designing bioactive molecules [1]. Among these, isoxazoles derivatives have been developed extensively due to their diverse synthetic methodologies and potential pharmacological activities such as antimicrobial, antifungal, anti-viral, anti-tubercular, anti-diabetic, anticancer, anthelmintic, antioxidant, anti-epileptic, antipsychotic, antimalarial, analgesic, anti-inflammatory etc [2-5]. Isoxazoles are the unsaturated five-membered heterocyclic aromatic compounds containing three carbon atoms, one oxygen atom, and one nitrogen atom in a ring system (**Fig. 1**) [6].

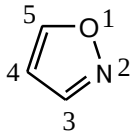

Fig. (1). Structure of isoxazole scaffold.

There are different types of clinically available drugs containing isoxazoles moiety that include Broxaterol (Bronchodilator), Acivicin (Antineoplastic agent), Cycloserine (Antibiotic), Risperidone (Antipsychotic), Danazol (Androgenic hormone), Valdecoxib (NSAID), Sulfamethoxazole (Antibiotic), Cloxacillin (Antibiotic), Flucloxacillin (Antibiotic), Ibetonic acid (Brain-lesioning agent), Leflunomid (disease modifying anti-rheumatic drug), edonentan (endothelin receptor antagonist) etc (**Fig. 2**) [7-9].

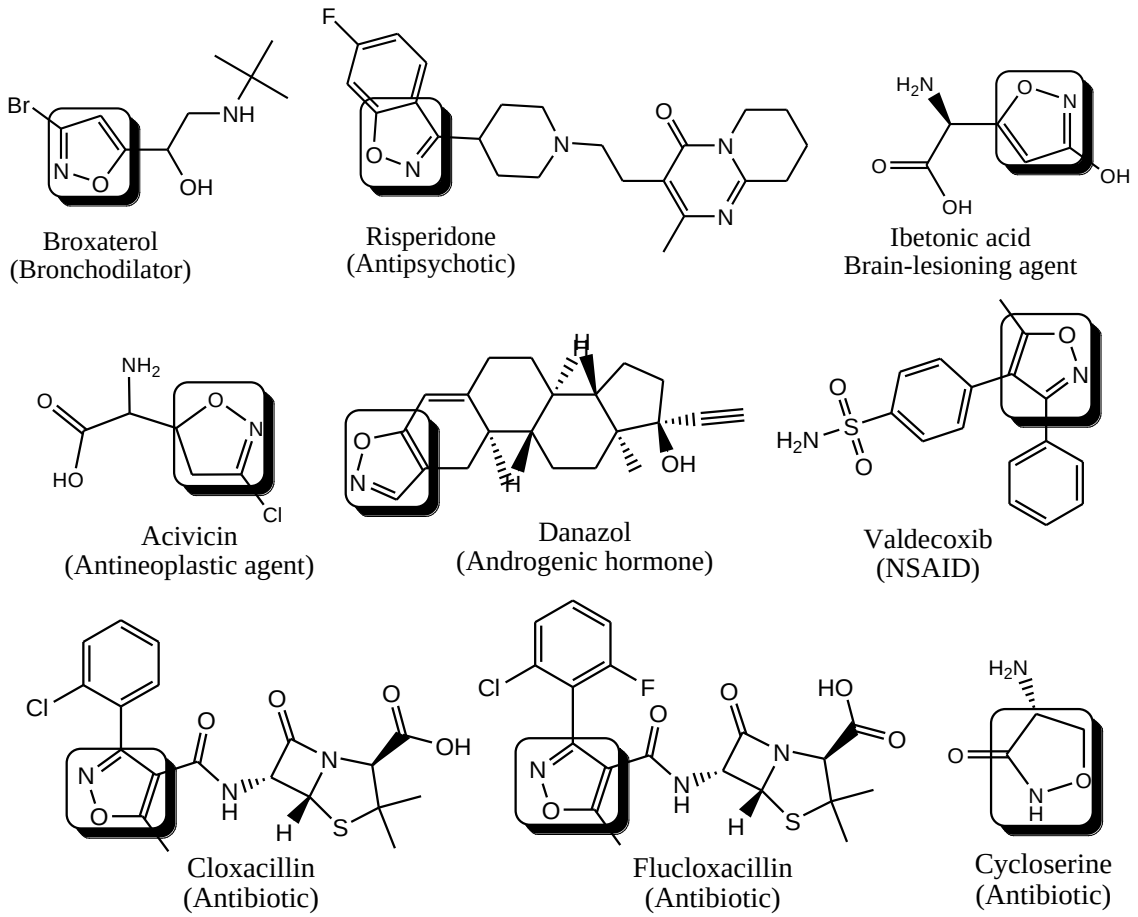

Fig. (2). Clinically available drugs containing isoxazole scaffold.

Similarly, chalcones are also playing significant role as building blocks for the synthesis of wide range of isoxazoles derivatives with diverse molecular structure *via* ring closure reactions [10]. Chalcones are the aromatic ketones with chemical name of 1,3-diarylprop-2-en-1-ones. The presence of α,β-unsaturated carbonyl system in the chalcone moiety makes these molecules more biologically active. Chalcones demonstrate diverse pharmacological properties including antioxidant, anti-inflammatory, antiviral, antidiabetic, antispasmodic, and antitumor etc (**Fig. 3**) [11-13].

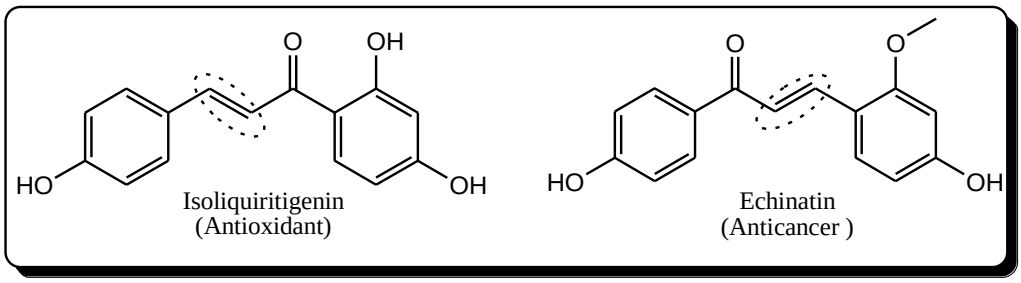

Fig. (3). Pharmacological properties of Chalcones.

Hence, a series of some novel isoxazole derivatives were synthesized *via* chalcones under microwave irradiation technique. This synthetic method is more advantageous as compared to conventional heating method in terms of selective heating, homogeneity, increased rate of reactions, improved product yield and cleaner reaction condition [14]. This heating technique plays major role to reduce the environmental pollution by using safer solvents, catalysts, suitable reaction conditions and there by increases the atom economy and energy efficiency of the synthetic process [15].

2. EXPERIMENTAL METHOD

The synthetic grade chemicals with high purity were used in the experiment. These were obtained commercially from S.D. Fine. Chem. Ltd. Mumbai, India. The synthesis of isoxazole derivatives was performed with the help of microwave oven that was working with power levels of 140-700W. The synthesis of isoxazole derivatives were performed at power level-2 with power level of 210W [22-24]. The melting points of the synthesized compounds were determined by using open capillary tubes and were found to be uncorrected. The completion of reaction was checked by TLC using silica gel as stationary phase and chloroform, ethyl acetate as mobile phase. The individual spots were visualized under ultraviolet lamp. FT-IR spectrometer (SHIMADZU) and ^{1}H-NMR (Brucker 400 MHz) spectrometer were employed for recording IR and ^{1}HNMR spectra respectively. The synthetic route for the title compounds (**4a-e**) is represented in figure **4**.

2.1. Synthesis of Chalcones

Synthesis of Chalcones is carried out based on Claisen-Schimdt condensation reaction. It involves the reaction between equimolar quantities of 2-hydroxy acetophenone (0.01mol) and substituted benzaldehydes (0.01 mol) in the presence of sodium hydroxide (NaOH) to afford corresponding Chalcones. TLC was monitored to confirm the completion of reaction. After completion of the reaction, the reacting materials were cooled on an ice bath to get the solid product of chalcones (**3a-e**).

2.2. Synthesis of Isoxazole derivatives

2.2.1. Conventional Synthesis

Equimolar mixture of chalcones (**3a-e**) (0.02 mol), hydroxylamine hydrochloride (0.02 mol) in ethanolic sodium hydroxide solution was refluxed on water bath for 1-2 h. TLC was monitored to check the completion of reaction. After completion of the reaction, the reaction mixture was kept on ice bath to yield the precipitate of isoxazole derivatives (**4a-e**). Finally, the product was filtered, washed with water, and dried.

2.2.2. Microwave induced synthesis

Equimolar mixture of chalcones (**3a-e**) (0.02 mol), hydroxylamine hydrochloride (0.02 mol) in ethanolic sodium hydroxide solution was refluxed under microwave irradiation at 210 W for 10-15 min. TLC was performed to check the completion of reaction. After completion of the reaction, the reaction mixture was kept on ice bath to obtain the precipitate of isoxazole derivatives (**4a-e**). Finally, the product was filtered, washed with water and dried.

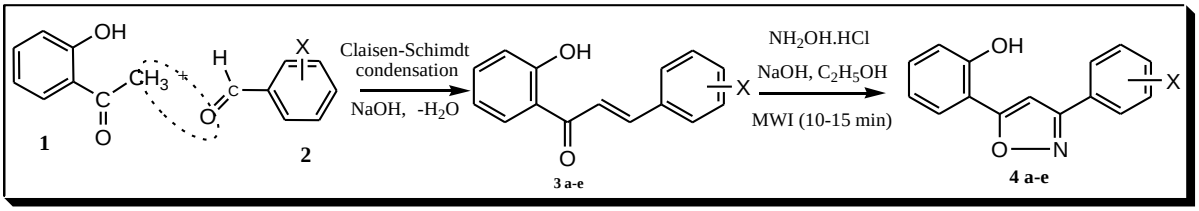

Fig. (4). Synthetic route of isoxazole derivatives (**4a-e**).

2.3. Antimicrobial Activity

The in vitro antimicrobial activity of the synthesized compounds were evaluated against different bacterial and fungal strains. The zone of inhibition of tested compounds was calculated in millimeters and was compared with the standard drugs. The anti-bacterial activity was screened based on cup plate method using bacterial strains including *Staphylococcus aureus* (MTCC 87), *Escherichia coli* (MTCC 40), *Staphylococcus epidermidis* (MTCC 2639), *Pseudomonas aeruginosa* (MTCC 424). The

test compounds were prepared in the concentration of 100 µg/ml using dimethyl sulphoxide (DMSO). The title compounds were tested by using nutrient agar as the medium. After 24 h of incubation at 37°C, the zone of inhibition formed were measured in mm against standard drug (Ampicillin). Similarly, the anti-fungal activity was carried out against different fungal stains such as *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 281). Ketoconazole was used as standard drugs for anti-fungal activity. DMSO was used as control for this activity study [16, 17].

3. RESULTS AND DISCUSSION

Various chalcones **(3a-e)** were synthesized by reaction of 2-hydroxy acetophenone with various substituted benzaldehydes in the presence of sodium hydroxide through Claisen-Schimdt condensation. Similarly, the synthesis of isoxazole derivatives **(4a-e)** was carried out *via* chalcones by cyclizaton of chalcones **(3a-e)** in the presence of hydroxyl amine hydrochloride and ethanolic sodium hydroxide solution. The yield of final product was improved in case of microwave heating methods (67-90%) as compared conventional synthesis (56-64%) as presented in table **1**. The characteristic spectra of α,β unsaturated carbonyl group of chalcone was observed near 1640-1650cm^{-1}. The IR spectrum of isoxazole derivatives exhibited absorption of λ_{max} for different 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 ^{1}H NMR spectrum, the aromatic protons were observed as multiplet at δ 6.86-8.56 and singlet at δ 6.46 for -OH. The mass spectra of the chalcone and isoxazole derivative exhibited molecular ion peak corresponding to their molecular formula. Compounds 4b, 4c and 4d displayed molecular ion peak at *m/z* 271.7, 271.7, and 255.24 respectively.

All newly synthesized isoxazole derivatives were evaluated for their in vitro antimicrobial activity at the concentration of 100 µg/ml and the results were compared with standard drugs (Table **2**). Among the tested compounds, compound 4b, 4d, and 4e exhibited significant activity as compared to standard drugs. The order of antibacterial activity in terms of zone of Inhibition (mm) was found to be **4d>4b>4e>4c>4a** and **4e>4d>4c>4b>4a** against *S. epidermidis* and *S. aureus* respectively. Similarly, the order of anti-fungal activity was found to be **4b>4d>4e>4c>4a** and **4c>4d>4e>4b>4a** against *C. albicans* and *A. niger* respectively.

Table 1. Comparative study on yield and reaction time.

Comp. code	X	R_f	M.P (^{0}C)	Conventional		Microwave	
				RT (min)	Yield (%)	RT (min)	Yield (%)
4a	H	0.54	148-150	60	64%	10	77%
4b	2-Cl	0.62	150-153	120	56%	12	72%
4c	4-Cl	0.63	155-157	90	63%	13	90%
4d	4-F	0.55	154-159	90	58%	12	67%
4e	4-OH	0.61	162-165	120	60%	10	78%

Table 2. Antimicrobial activity of synthesized compounds (4a-e).

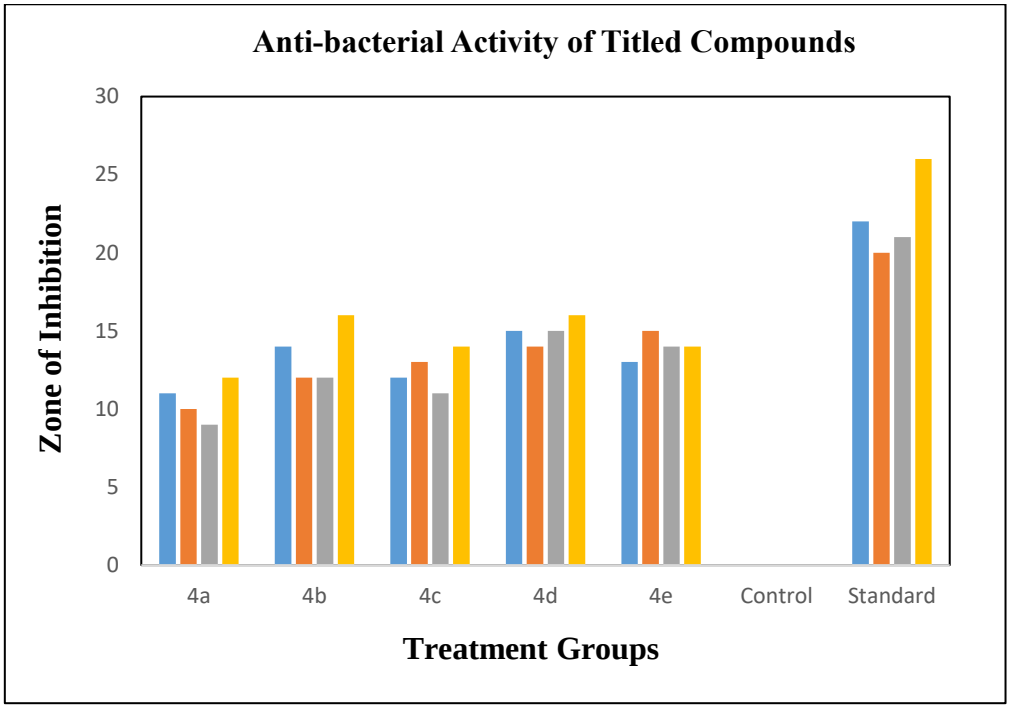

Fig. (5). Anti-bacterial Activity of Titled Compounds (4a-e).

Structure activity relationship (SAR) Study

The presence of α,β-unsaturated carbonyl system on chalcone moiety exhibits significant biological activity. Similarly, the presence of electron withdrawing group like chloro, flouro, and hydroxyl on isoxazole ring exhibits better activity as compared to the other derivatives. The presence of aryl ring is found to increases the lipophilic property of the molecule. Further, the presence of hydroxyl group acts as hydrogen bonding domain [18].

Compounds (100µg/ml)	**Zone of Inhibition (mm)**					
	Antibacterial activity				**Anti-fungal activity**	
	Gram positive		Gram negative			
	S. epidermidis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
4a	11	10	9	12	7	8
4b	14	12	12	16	11	9
4c	12	13	11	14	8	12
4d	15	14	15	16	10	11
4e	13	15	14	14	9	10
Control	-	-	-	-	-	-
Standard	22	20	21	26	16	15

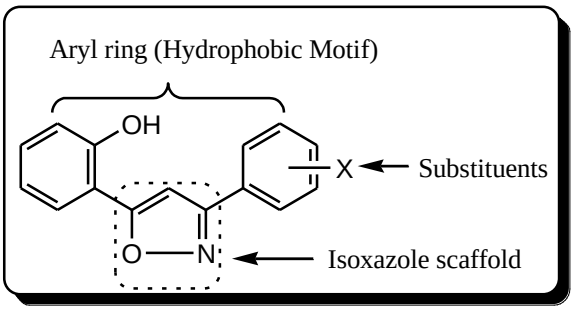

Fig. (5).SAR Study of Isoxazole derivatives.

Conclusion

A variety of novel isoxazole derivative were synthesized via chalcones under microwave irradiation with good yield in a short period of time as compared to conventional heating method. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, Mass spectroscopy. Further, the titled compounds were evaluated for their anti-microbial activity. From the screening results, it was revealed that the tested compounds exhibited significant anti-microbial activity as compared to the standard drugs. Moreover, compounds with electron withdrawing group displayed remarkable antimicrobial activity than the compounds with electron releasing group. Further, the structural modification of isoxazole scaffold is needed to generate safer and potential therapeutic agents.

LIST OF ABBREVIATIONS

^{1}H NMR = Proton Nuclear Magnetic Resonance
Comp. = Compounds
Cl = Chloro
DMSO = Dimethyl sulfoxide
ºC = Degree centigrade
F = Fluro
I.R = Infrared Spectroscopy
KBr= Potassium Bromide
M.p. = Melting point
min. = Minute
mL = Milli liter
mm= Milli miter
MWI = Microwave irradiation
NaOH = Sodium hydroxide
ppm= Parts per million
R_f= Retention factor
OH = Hydroxyl
TLC = Thin Layer Chromatography
µg = Micro gram
W = Watt

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Not applicable

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflictof interest.

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