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GREEN SYNTHESIS, CHARACTERIZATION & BIOLOGICAL EVALUATION OF 3,5-DIPHENYL ISOXAZOLE DERIVATIVES

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Abstract

3,5-Diphenyl Isoxazole derivatives, constituting an important family of five-membered heterocycles with one oxygen atom and one nitrogen atom at adjacent positions is of immense importance because of its wide spectrum of biological activities and therapeutic potential. It is, therefore, of prime importance that the development of new synthetic strategies and designing of new isoxazole derivatives should be based on the most recent knowledge emerging from the latest research. This review is an endeavour to highlight the progress in the chemistry and biological activity of 3,5-Diphenyl isoxazole derivativesto the medicinal chemists for the development of clinically viable drugs using this information. One series of total 10 (3,5-Diphenyl Isoxazole) derivatives were synthesized but Compound A, Compound H & Compound I were designed elemental analysis and evaluated for antifungal and antioxidant activity.

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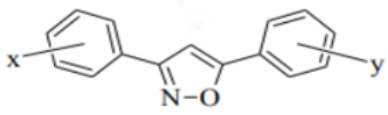
Introduction

3, 5-Diphenyl Isoxazole is a heterocyclic compound with a five-membered ring that has oxygen and nitrogen atoms at the 1 and 2 positions, and their partially saturated analogues are known as isooxazoline. Many biologically active products contain derivatives of these heterocyclic compounds. Derivatives containing isoxazole fragments possess biological activities such as anticancer, anti-inflammatory, antibacterial, anti-Alzheimer's disease, antioxidant, insecticidal, antifungal, and antidiabetic. Isooxazolines and isoxazoles have unique electronrich aromatic structures and have received much attention. The photolysis of isoxazole was first reported in 1966 [1-6]. Due to the weak N-O bond, the isoxazole ring tends to collapse under UV irradiation, rearranging to oxazole through azirine intermediate. Meanwhile, the azirine intermediate can react with nucleophiles, especially carboxylic acids. Given the photoreactions, isoxazole group is developed as a native photo-cross-linker for photoaffinity labelling and chemo proteomic studies.

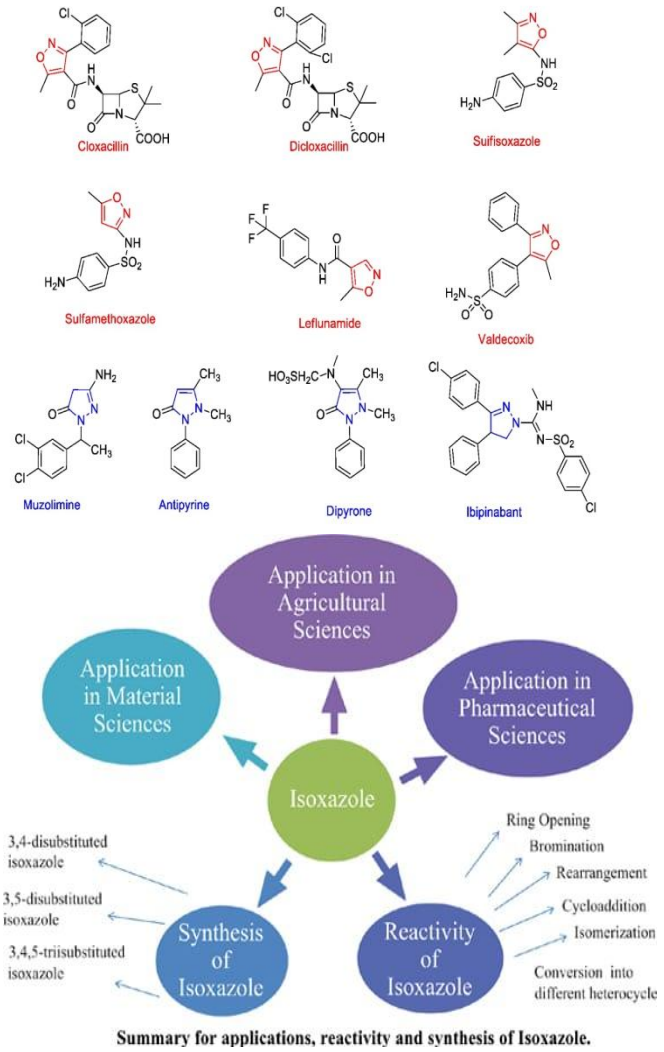


STRUCTURE OF 3, 5 DIPHENYL-ISOXAZOLE DERIVATIVES

Therapeutic Properties

Derivatives containing isoxazole fragments possess biological activities such as anticancer, anti-inflammatory, antibacterial, anti-Alzheimer's disease, antioxidant, antifungal and antidiabetic.

Medicines containing 3,5-diphenyl isoxazole moiety available in the market are :Valdecoxib(anti-arthiritis), dicloxacillin (penicillin antibiotic), leflunamide(rheumatoid arthiritis), ibipinabant(trement of obesity) ,sulfisoxazole(sulpha drug)



Summary for applications, reactivity and synthesis of Isoxazole.

Green Synthesis

Microwave radiation, an electromagnetic radiation, is widely use as a source of heating in organic synthesis. The basic mechanisms observed in microwave assisted synthesis are dipolar polarization and conduction. Microwave assisted organic synthesis (MAOS) has emerged as a new "lead" in organic synthesis. The technique offers simple, clean, fast, efficient, and economic for the synthesis of a large number of organic molecules, have provided the momentum for many chemists to switch from traditional heating method to microwave assisted chemistry. Microwaves heat the compounds directly therefore usage of solvents in the chemical reaction can be reduced or eliminated.

Benefits Of Microwave Assisted Synthesis

- Faster reaction
- Better yield and high purity
- Energy saving
- Uniform and selective heating
- Reproducibility

Aim & Objectives

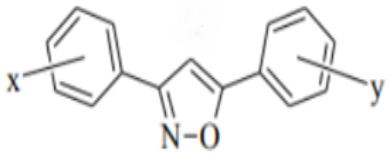
Isoxazole having nucleus having better therapeutic effective and successfully used in clinical practice.so our main objective is to synthesize novel derivatives containing isoxazole nucleus having better therapeutic activity with new tangents, low cost, superior pharmacokinetic activity and minimum side effects.

- **SIGNIFICANCE OF THE STUDY**Research had been carried out on Green synthesis, characterization and biological evaluation of 3,5-Diphenyl isoxazole derivatives, proving to have a significant antioxidant and antifungal activity

SCOPE OF THE STUDY

Novel Green synthesis, characterization and biological evaluation of 3,5-Diphenyl isoxazole derivatives will provide defined scope for researches for the advancement of novel potential drug candidates have better efficacy and activity

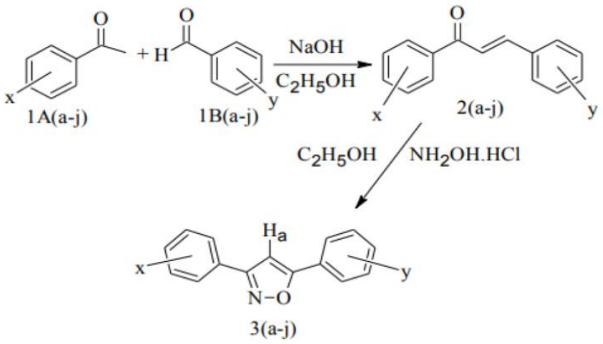
SCHEME -1



Plan of Work

To about 5 ml of ethanol and 7ml of 10% NaOH (aq) taken in a 50 ml microwave RBF, equimolar amount of substituted acetophenone(0.05mmol) was added followed by the addition of substituted benzaldehydes(0.05mmol). The mixture was synthesized in microwave for 5 minutes at a power level of 350 watt and kept in a deep freeze for an overnight. The solid products 1,3-diaryl prop2-ene-1-one (2a-j) so formed were neutralized by hydrochloric acid. The products were filtered off and recrystalized by ethanol. After that in a 50ml RBF equimolar of hydroxylamine hydrochloride and compounds (2a-j) were mixed in presence of 5 ml of ethanol and kept in the microwave for 5 mins at a power level of 350 watt. The mixtures were kept in deep freeze for an overnight. The solid products 3,5-diarylisoxazoles (3a-j) so formed were neutralized by 1%sodium hydroxide solution. The product were filtered off and recrystallized by ethanol. The completion of the reaction was monitored by TLC.

Compound	Molecular Formula	Molecular Weight	M.P	R.F	% Of Yield
Scheme 1 A	$C_{15}H_{11}NO$	221	142 ®c	0.54	94 %
Compound **H**	$C_{15}H_{11}NOBrCl$	336	140 ®c	0.57	84 %
Compound **I**	$C_{15}H_{11}N_2O_2Br$	331	139 ®c	0.77	85 %



Derivatives of Synthesized Compounds

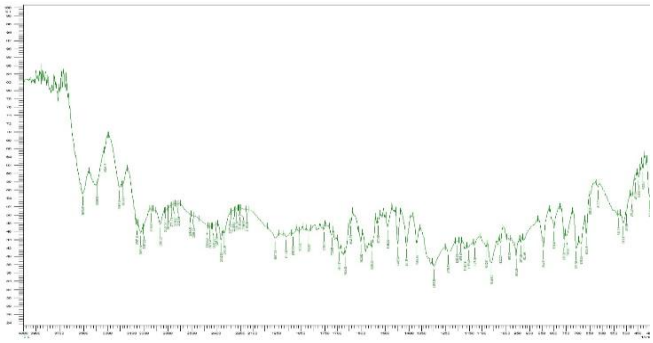
Compound	X	Y
A	**H**	**H**
B	H	p-Br
C	H	p-F
D	H	m,p-Cl
E	H	m,p- NO_2
F	H	m,p-Cl
G	H	m,p-OCH_3
H	**p-Br**	**p-Cl**
I	**p-Br**	**p- NO_2**
j	p-Cl	m,p-OCH_3

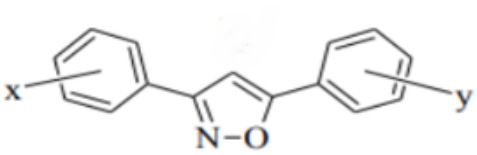
Material and Method

The melting temperature of the pure drug 3,5-diphenyl Isoxazole derivatives utilized in the Synthesis, which was acquired from Loba Chemie, was determined to be 142®C. analytical- grade materials were utilized for all compounds and reagents. The uncorrected melting points were established using open capillaries and an electro thermal device. On precoated 0.2-mm silica gel G60 F254 plates, thin-layer chromatography were performed (Merck). The reaction were monitored using precoated TLC silica gel F_{254}aluminium plates and solvents (choloroform:methanol:1:1) used as mobile phase .IR spectra were recorded on a Shimadzu-Fourier transform infrared (FT-IR)-8400 Spectrophotometer using KBr disc.

Physical Characterization of Synthesized Compound

IR Spectroscopy of 3, 5-Diphenylisoxazole Derivatives





Interpretations of IR Spectroscopy

FTIR (KBr, cm^3) 3057.17(Aromatic C-H stretching), 1556.55 (C-N stretching), 1490.97(C-C stretching), 1411.89 (N-O stretching), 923.90 (C-C stretching), 688.59 (Monosubstituted C-H def.)

COMPOUND A : 3,5-Diphenylisoxazole : Yield 94%,M.P. 142ºC,FTIR(KBr, cm^3) 3057.17(Aromatic C-H stretching), 1556.55 (C-N stretching), 1490.97(C-C stretching), 1411.89 (N-O stretching), 923.90 (C-C stretching), 688.59 (Monosubstituted C-H def.) ,^{1}HNMR(DMSO)δ 6.562(s,1H,=CH) 7.342-7.826ppm(m,10H,Ar-H),ESI-MS:m/z (%) 221.24[100]

Biological Evaluation of Synthesized compound

Anti-Fungal Activity

Disk diffusion assay

Agar disk diffusion assay has been utilized flexible, cost-effective and more accurate method for determining antifungal susceptibility against the strain of fungus and yeast. This method was developed in the year of 1940 and is successfully used in much clinical microbiology approved by CLSI for microorganism and well standardized. For the best studies of antifungal activity through agar disk diffusion assay is a disk of clotrimazole against *Candida albicans* gives a promising result. The assay is performed by applying microbial inoculums of approximately 1-2 × 10^8 CFU/mL to the 150 mm of the solid agar plate. Antimicrobial compounds at various concentrations containing ~ 6 mm diameter of filter paper are placed over the agar solid surface. The Petri dishes containing both microbes and antimicrobial compounds were incubated under optimized conditions. To check the best reliable result for antifungal activity, the optimization incubation period was approximately 4–7days at 30°C for fungal growth . The zone diameter around each tested compound proposes that inhibition of growth of the fungal strain is assessed by the nearest millimetre. The rate of the susceptibility of microbes and diffusion of drugs on the agar plate denoted by the diameter of zone formation surrounding the attached disk. The obtained results through the disk diffusion method constituted a good model to be used for designs the analysis for other fungal strain and drug as well. Research reported also indicate disk diffusion studies of micafungin against *Candida albicans Streptomyces gresus, Aspergillus Niger, Aspergillus fumigalis*

As a result, there is an increasing need to design new ant antifungal agents with better activity profile and lower toxicity.

MINIMAL FUNGICIDAL CONCENTRATION (MFC)(µg/ml)

Sl.no	MICRO ORGANISM	Zone of Inhibition Compound (10µg/disc)										STANDARD CLOTRIMAZOLE (10µg/DISC)
		C-GY	C-GU	C-PA	C-TY	C-CY	C-HS	C-TP	C-PT	C-T	C-HH	
1	*Candida albicans*	6	5	-	3	3	-	4	3	3	-	6
2	*Streptomyces gresus*	4	5	3	4	-	5	4	-	-	-	7
3	*Aspergillus Niger*	5	-	4	3	8	5	6	4	-	-	5
4	*Aspergillus fumigalis*	4	7	3	5	3	-	3	6	-	-	5

Methods used for primary and secondary screening.

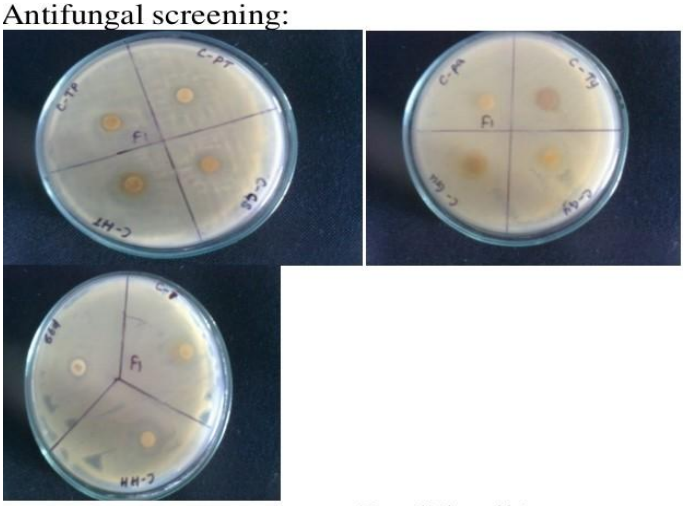
Primary screen: In primary screening 1000 micro/ml concentration of the synthesized drug were taken.

Secondary screening: The active primary screening were diluted to get 500 micro/ml, 250 micro/ml 125 micro/ml and 62.5 micro/ml DMSO was utilized as a diluent.

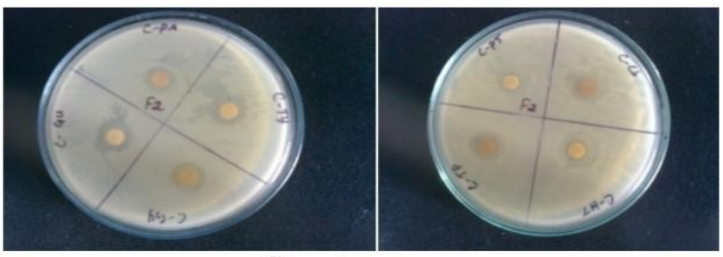
From the result obtained for antifungal screening for the synthesized compound it showed that 2,3-diphenyl isoxazole derivatives exhibits moderate to good antifungal activity against microorganism, with zone of inhibition range of 3-8mm.

In that compound C-HH,C-T showed good activity with the zone of inhibition in the range of 8mm at 62.5 micro/ml.

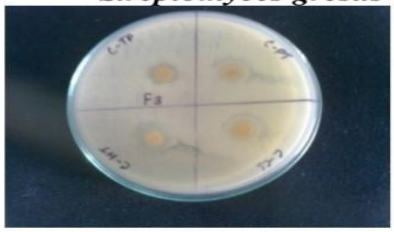
Antifungal screening:



Candida albicans



Streptomyces gresus



Aspergilus niger

Aspergilus fumigalis

Antioxidant Activity

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H- A) can be written as,

(DPPH) + (H-A) DPPH-H+(A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure

Different Concentration of synthesized products (220µl) was made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the synthesized product was calculated using the following formula,

% RSA = Abs control Abs sample Abs control x 100

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol, Abs sample is the absorbance of DPPH radical + Synthesized drug

Calculation of % Radical Scavenging Activity & IC50 from DPPH assay

Concentration (µg/ml)	control	sample	% RSA	IC50
50	0.52	0.312	40.00	2.34
100	0.52	0.291	44.04	7.73
150	0.52	0.228	56.15	13.12
200	0.52	0.18	65.38	18.51
250	0.52	0.14	73.04	23.90
300	0.52	0.075	85.58	29.29
350	0.52	0.035	93.27	34.64

Ascorbic acid 0.014 0.058 82

The % radical scavenging activity of the synthesized product.

The in vitro antioxidant properties of the newly synthesized compound at different concentrations were examined by a well documented assay like DPPH free radical Scavenging assay. The results indicate that newly synthesized compound a showed good antioxidant activity at low concentrations as compared to standard drug.

Results and Discussion

The aim of the undertaken research work was to make an effort in the direction of synthesizing molecules, which could have the capability of antifungal activity at 125 micro/ml. The title compound was synthesized as depicted in the scheme 1.The synthesized compound was subjected to Physicochemical Characterization. The sharp Melting points, TLC and spectral analysis confirmed the purity and homogeneity of title compound. The synthesized compound was obtained in the solid state in the yield 94%.The IR spectra and NMR spectra of the scheme 1 confirmed by anticipated structure.

The compounds synthesized were evaluated for their antifungal activity against *Candida albicans Streptomycesgresus, Aspergillus Niger, Aspergillus fumigalis*. From the result obtained for antifungal screening for the synthesized compound it showed that 2, 3-diphenyl isoxazole derivatives exhibits moderate to good antifungal activity against microorganism, with zone of inhibition range of 3-8mm.

In that compound C-HH, C-T showed good activity with the zone of inhibition in the range of 8mm at 62.5 micro/ml at different concentrations were examined by a well documented assay like DPPH free radical Scavenging assay. The results indicate that newly synthesized compound A showed good antioxidant activity at low concentrations as compared to Ascorbic acid.

Conclusion

One series of total 10 (3,5-Diphenyl Isoxazole) derivatives were synthesized but Compound A, Compound H & Compound I were designed elemental analysis and evaluated for antifungal and antioxidant activity. Characterization like Melting point, TLC, IR, NMR spectra of synthesized compounds (Compound A) were conducted. From the result obtained for antifungal screening for the synthesized compound it showed that compound A was found highly antifungal activity at 125 micro/ml. The results of antioxidant activity indicate that newly synthesized compound A showed good antioxidant activity at low concentrations as compared to standard drug Ascorbic acid.. In conclusion, 3,5-diphenylisoxazole radicals play an important role in the synthesis of many drugs and have drawn considerable interest from researchers.

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Conflict of Interest
The authors declare No conflict of interest.

Ethical Statement
Not Applicable.

Author Contribution
All authors are contributed equally.

Informed Consent
Not required

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