RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2661-2665| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SYNTHESIS AND ANTI-INFLAMMATORY, ANTIOXIDANT STUDIES OF NOVEL CHALCONES-BASED ISOXAZOLE DERIVATIVES

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ABSTRACT
Our previous work identified various novel Chalcones (I-III) produced by mixing the equimolar amount of Ferrocene aldehyde and different substituted acetophenones which has a strong base. Hence, in the present study, novel various Chalcones (I-III) were treated with hydroxylamine hydrochloride to form various Isoxazoles (Ic-IIIc). The synthesized novel chalcone-based Isoxazole derivatives were determined by spectroscopic techniques such as^{1}H,^{13}C-NMR, FT-IR,and UV. Further, these products were evaluated for antimicrobial studies and antioxidant susceptibilities through DPPH radical scavenging method. The synthesized isoxazole (Ic) was subjected to a study of their anti-inflammatory activity(COX and LOX) assay.
Keywords: Substituted Acetophenones, Ferrocene Aldehyde, Chalcones, Hydroxylamine Hydrochloride, Isoxazole.
RASĀYAN *J. Chem.*, Vol. 15, No.4, 2022

INTRODUCTION

The compound known as Chalcones is 1, 3-diphenyl-2-propene-1-one, which contains two aromatic rings, connected with a three-carbon α,β-unsaturated carbonyl system.[1,2] Aldehydes and ketones are combined by a process called Clasien-Schmidt condensation, which is then followed by dehydration to produce chalcones.[3] Nitrogen-containing heterocyclic compounds are greatly important to both medical and organic chemists, and their synthesis continues to represent challenges from pharmaceutical perspectives.[4]Organ metallic chemistry is gaining popularity, especially in recent decades, due to its numerous biological and medical uses, which spearheads innovative region termed bio-organometallic chemistry.[5]The ferrocene moiety is employed in "bio-organometallic" chemistry because of its stability, physical activity, and utility in the organic synthesis to create new compounds.[6]One of the most researched and created molecules in the field of medicine is chalcone, which may be either synthetic or naturally occurring.[7]The compound with the backbone of Chalcones was reported to exhibit a wide variety of pharmacological activities including antimalarial[8], antibacterial[9], antitumor[10], anticancer[11],anti-inflammatory[12], antifungal[13], antioxidant[14], antileishmanial[15],cytotoxic activity.[16] The present work describes the synthesis of Chalcones using various acetophenone moieties and ferrocene aldehydes. Further, this study extended to the synthesis of its isoxazole derivatives. All the synthesized compounds are characterized by spectral analyses of ^{1}H, ^{13}C-NMR, FT-IR and UV. Antibacterial, antifungal, and antioxidant activities were tested on the produced compounds. The synthesized 5-(4ˈcholrophenyl)-4-(dicyclopentadienyliron)-1,2-Isoxazole were evaluated for their anti-inflammatory activity (COX and LOX) assay.

EXPERIMENTAL

All the chemicals were provided by Sigma Aldrich. Melting point determination is conducted using digital melting point apparatus. JASCO UV spectrophotometer was used to measure UV-Vis spectra. Fourier transform-Infrared spectroscopy (Perkin Elmer) was used to record the infrared spectrum. A Bruker device was employed to note the proton NMR spectra at 400MHz and carbon-13 NMR spectra at 100MHz. The Synthesis and Spectral Characterization of Chalcone Compounds (I-III) has been published

http://doi.org/10.31788/RJC.2022.1547023

previously.[17]

General Procedure for the Synthesis of Isoxazole Derivatives (Ic–IIIc)

The synthesis of isoxazole is depicted in Fig.-1 (Ic-IIIc). In ethanol (25 mL), a mixture of Chalcones(I,II,III) (0.02mol), hydroxylamine hydrochloride (0.02), and sodium acetate were mixed and refluxed for 8hrs before being placed in 400mL of water, and refrigerated overnight. Prior to recrystallization from ethanol, the filtrate was washed with distilled water.[18] A melting point of the digital device was utilized to measure melting points, and TLC was employed to analyze the completion of the reaction.

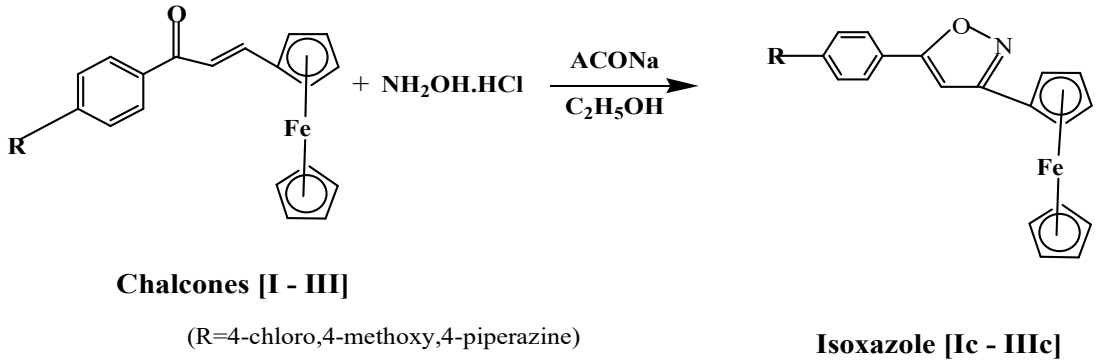

Fig.-1: Synthetic Representation for the Synthesis of Isoxazole(Ic-IIIc)

Spectral Studies of Isoxazole (Ic-IIIc)

5-(4'-chlorophenyl)-4-(dicyclopentadienyl iron)-1,2-Isoxazole [Ic]

Yield: 84%.M.P.: 158ºC. Mo.F: $C_{19}H_{14}FeClNO$. %found (calcd): C-62.86 (62.83), H-3.88 (3.85), N-3.87 (3.85); O-4.45 (4.40). Ultraviolet (λ_{max}, nm):475nm corresponds to n→π* shift. IR (KBr, υ_{max}, cm^{-1}): 489 (Fe-cp), 1401 (Ar-C=C), 1357 (C-O), 862 (C-Cl), 1655(C=N). ^{1}H NMR ("400MHz, $CDCl_3$"): 7.3 (d, 2H, 2CH, J-5.4Hz), 6.54 -7.9 (m, 8H, Ar-H), 4.1 (s, C-Cl),7.75-7.79(d,2H,2CH, J-6.6Hz). ^{13}C NMR ("100MHz, $CDCl_3$"): 66.18-77.85(C-O),77.85(C-N), 114.37-169.81 (aromatic compounds).

5-(4'-methoxyphenyl)-3-(dicyclopentadienyl iron)-1, 2-Isoxazole[IIc]

Yield: 86%. M.P.: 167°C. Mo.F: $C_{20}H_{17}FeNO_2$. %found (calcd): C-66.82 (66.85), H-4.84 (4.73), N-8.82 (8.91); O-3.6 (3.9). Ultraviolet (λ_{max}, nm): 455nm corresponds to n→π* shift. IR (KBr, υ_{max}, cm^{-1}): 486 (Fe-cp), 1439 (Ar-C=C), 1332 (C-O), 2853 (C-OCH_3), 1648 (C=N).^{1}H NMR ("400MHz, $CDCl_3$"): 7.0(d, 2H, 2CH, J-5.2Hz), 7.26-7.99 (m, 10H,Ar-H), 3.8(s,3H,OCH_3), 7.75 (d, 2H,2CH, J-6.4Hz).^{13}C NMR("100MHz, $CDCl_3$"):55.40(OCH_3),77.35(C-O),7.08-69.97(C-N),114.27-169.41(aromatic compounds).

5-(4'-piperazinephenyl)-4-(dicyclopentadienyl iron) -1,2-Isoxazole [IIIc]

Yield:84%. M.P.: 170°C. Mo.F: $C_{23}H_{23}FeN_3O$. %found (calcd): C-66.89(66.85), H-5.62 (5.57), O-3.92(3.87); N-10.22(10.17). Ultraviolet (λ_{max},nm): 408nm corresponds to n→π* shift. IR (KBr, υ_{max},cm^{-1}): 485 (Fe-cp), 1441 (Ar-C=C), 1390 (C-O), 1611(C=N).^{1}H NMR ("400MHz, $CDCl_3$"): 7.02 (d, 2H,2CH, J-8Hz), 7.4-7.9 (m, 13H, Ar-H), 7.76(d, 2H,2CH,J-4Hz), 3.8(s, 1H, Aliphatic NH). ^{13}C NMR("100MHz, $CDCl_3$"): 68.92(C-N), 112-169.64 (aromatic compounds).

RESULTS AND DISCUSSION

The set-up technique is straightforward, and the resulting yield is great. Spectral as well as elemental analytical (Ic-IIIc) data were used to verify the structure of substances. The absorption peak of UV-Vis

can be detected in UV-Visible absorption spectra. The red-shift absorption (n→π* transition) maxima of Isoxazole (Ic-IIIc) were 475, 455, and 408nm respectively owing to their extended conjugation. The band was seen at 1357, 1332, and 1330 cm^{-1}, which validated the C-O stretching mode of the produced Isoxazole molecule. Bands of absorption for "functional groups" like -NH,-OCH, and -Cl were seen at 1545cm^{-1}, 2853 cm^{-1},and 862 cm^{-1}. For C=N stretching, an absorption band at 1655, 1648, and 1611cm^{-1} was found. At 7.2,7.0,and 7.02, the (Ic-IIIc) doublet was found in the ^{1}H-NMR spectrum with significant J value of 5.4, 5.2 and 8.0 Hz. The subsequent detection of multiplets at 7.0–7.9 confirmed the cyclization of isoxazoles(Ic-IIIc). Then a singlet appeared at 4.1 for the chloro group as well as 3.8 for the Methoxy group. The carbonyl carbon appeared between 114.27 and 169.41 in the ^{13}C NMR spectrum for compounds (Ic-IIIc).

Antibacterial Activities

The antibacterial and antifungal studies of Chalcone compounds (I-III) have been published in A.Suyambulingam *et al.*[17] The antibacterial activity of the drugs developed is shown in Table-1. *Invitro* tests on antibacterial activity were conducted on the gram-negative *Klebsiella pneumonia* MTCC530, *Proteus Vulgaris* MTCC426) and gram-positive (*Bacillus subtillis* MTCC113, streptococcus *mutants* MTCC916)bacteria. The minimum inhibitory concentration was found and evaluated to traditional antibacterial drugs like *streptomycin*. The antibacterial studies of Isoxazole compounds (Ic-IIIc), and compound (Ic) showed high activity compared to standard drugs. Compound (IIc) was moderately active toward *Klebsiella pneumonia, Bacillus subtillis,* and *Proteus Vulgaris*; it was inactive with *streptococcus mutants*. The compound (IIIc) Klebsiella pneumonia was moderately active towards *Proteus Vulgaris* and *streptococcus mutants*; it was inactive *Bacillus subtillis.*

Table-1: Antibacterial Compounds (Ic-IIIc) Data

Compound no.	Minimum inhibition concentration in millimeter(mm)			
	gram +ve	*gram +ve*	*gram -ve*	*gram-ve*
	Streptococcus mutans	*Bacillus subtillis*	*Klebsilla pneumonia*	*Proteus vulgaris*
Ic	8	9	7	8
IIc	---	9	8	9
IIIc	8	---	10	9
PC	20	21	20	20
NC	---	---	---	---

PC (*streptomycin*) Positive Control, NC (no antibiotics) Negative Control (plain disc)

Antifungal Activities

The antifungal activity of the drugs developed is shown in Table 2. Three different fungi strains-*Rhizopus stolonifera* MTCC162, *Aspergillus flavus* MTCC535, and Aspergillus *niger* MTCC281were investigated for antifungal activity. The minimum inhibitory concentration was found and compared to traditional antibacterial medicines like *fluconazole*.

Table-2: Antifungal Data of Compounds (Ic-IIIc)

Compound no.	Minimum inhibition concentration in mm		
	Fungal strains		
	Aspergillus flavus	*Aspergillus niger*	*Rhizopus stolonifer*
Ic	26	8	7
IIc	9	9	8
IIIc	---	---	7
PC	15	18	20
NC	---	---	---

NC-Negative Control, PC-Positive Control (*fluconazole)* (Plain Disc)

Antifungal activity of Isoxazole (Ic-IIIc),and Compound (Ic) showed high activity compared to standard drugs. Compound (IIc) showed moderate activity with *Aspergillus niger, Aspergillus flavus,* and *Rhizopus stolonifer.* Compound (IIIc) was moderately active towards *Rhizopus stolonifer*; it was inactive with

Aspergillusniger and *Aspergillus flavus.*

The Activity of DPPH Radical Scavenging

Preliminary evaluation findings showed that among the produced compounds (Ic- IIIc), Compounds(Ic),(IIc), and (IIIc) showed IC_{50} values at 215.274, 221.031,and 325.803µM respectively when compared to that of the standard ascorbic acid at 393.486µM. However, Compound (Ic) demonstrates high activity, its IC_{50} value 215.275µM, when compared to standard ascorbic acid at 393.486µM, which comprise electron-donating substituent like chlorine Pharmacophore, particularly on the carbon-4 position of "aromatic ring-B" showed good properties of antioxidant. Table-3 summarizes the antioxidant activity findings.

Table-3: Antioxidant Activity DPPH Method

Sample	Concentration			IC_{50} Value
	100 µg/ml	200µg/ml	300µg/ml	
I c	40.483 ± 0.010	46.400 ± 0.022	58.898 ± 0.022	215.274
IIc	41.972 ± 0.020	45.819 ± 0.157	57.356 ± 0.008	221.031
IIc	45.819 ± 0.157	47.882 ± 0.022	49.449 ± 0.068	325.803
Standard (Ascorbicacid)	24.746 ± 0.020	33.291 ± 0.011	41.971±0.029	393.486

Anti-inflammatory Assay

This study, evaluated anti-inflammatory activity only in compounds (Ic), with a compound with good antioxidant activity. Synthesized Isoxazole(Ic) was evaluated in two assays on anti-inflammatory activity COX and LOX assay. Cyclooxygenase (COX) activity, compound (Ic) showed IC_{50} value at 84.03µM when compared to that of the standard *diclofenac* at 42.38µM. Lipoxygenase(LOX) activity, compound (Ic) showed IC_{50} value at 96.83µM when compared to that of the standard *diclofenac* at 45.85µM.It was noticed that the compound (Ic), that chloro substitution on the aromatic ring showed more inhibition.

CONCLUSION

A series of Isoxazole with antibacterial, antifungal, and antioxidant activity was produced using a standard technique from cyclization of Chalcones. The antimicrobial activity of Isoxazole compounds (Ic-IIIc) was excellent. The antioxidant activity of the compound (Ic) was excellent. Synthesized Isoxazole(Ic) was tested for anti-inflammatory activity COX and LOX, it showed moderately active when compared to the standard *diclofenac*.

ACKNOWLEDGEMENT

The authors appreciate the laboratory facilities provided by the Head, Department of Chemistry, SAC, Nagercoil. The authors also acknowledge Gandhigram University for providing the IR and NMR spectrum analyses. For Microbial investigations, we would like to express our gratitude to Inbiotics, Nagercoil.

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[RJC-7023/2022]