A Conventional and Microwave Assisted Synthesis of 4,6- Diphenyloxazines and Its Characterisation

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Abstract**: In a first stage substituted chalcone derivatives were synthesized by a conventional and microwave assisted reaction of some substituted benzaldehyde with substituted acetophenone by a Claisen-Schmidt condensation using PEG-400 as solvent by green chemistry approach. In a second stage substituted chalcone derivatives were allowed to react with urea in the presence of sodium hydroxide in ethanol as catalyst which gives oxazine as product by a both methods of conventional and microwave irradiation. The progress of the reaction was monitored by TLC and separated the compound using column chromatography. The target compounds were subjected to various characterization viz.,UV, FT-IR, 1H-NMR and Mass spectra. The anti-microbial activities of compounds have also been tested using Minimum Inhibitory concentration (MIC) method with two different microorganisms (*Staphyloccocous aures* (MTCC3381), and *Escheriochia coli* (MTCC739). The results of the antimicrobial activity clearly shown that the 4,6-diphenyl substituted oxazine derivatives have moderate inhibiting nature against both types of bacteria than corresponding chalcones. (Gram-positive and Gram-negative).**

KeyWords: Chalcones; Oxazine; antimicrobial activity; PEG–400; Urea; MWI

I. Introduction

Chalcones were prepared by condensation of acetophenone with aromatic aldehydes in presence of suitable condensing agent[1]. They undergo a variety of chemical reactions that leads to many heterocyclic compounds[2,3]. Chalcones have been used as intermediates for the preparation of compounds having therapeutic value[4]. Many reviews reveal that chalcone derivatives exhibit diverse pharmacological activities, such as potential cytotoxic agents, antimicrobial agents, antiviral, anti-inflammatory, anesthetic, etc.[5,6]. In the view of the varied biological and pharmacological applications, we have planned to synthesize some heterocyclic derivatives of chalcone and test their antibacterial activity

Many heterocyclic analogous of chalcones have been synthesized and subsequently demonstrated to possess biological and pharmacological activities, which may possibly result in chemotherapeutic agents. Because of great potentiality the heterocyclic analogous of chalcones are most helpful synthons. In the view of the varied biological and pharmacological application, we synthesized some heterocyclic derivatives of chalcones1. In recent years, attention has increasingly been given to the synthesis of oxazine derivatives as a source of new antimicrobials.

The oxazines were medicinally important due to the presence of oxygen, nitrogen heteroatoms along with a double bonds in their structural moieties [7]. The important medicinal activities of these oxazines are anti-bacterial [7-8], anti-fungal [7-8], anti-plasmodial [9], anti-cancer [10], anti-depressants [11], anti-osteoplastic [12], anti-tumour [13], anti-oxidant [14], anti-tuberculosis [15], anti-neoplastic [16], antagonists [17], anti-inflammatory [18] agents.

Reducing or eliminating the use of volatile organic solvents can minimize the generation of waste, which is a requirement of one of the principles of green chemistry. Recently, polyethylene glycol (PEG) has been found to be an interesting solvent system. Based on the careful analysis of the literature, present investigation focused on the PEG-400 mediated synthesis of chalcone which on further cyclization with urea resulting 4,6- diphenyl substituted oxazine.

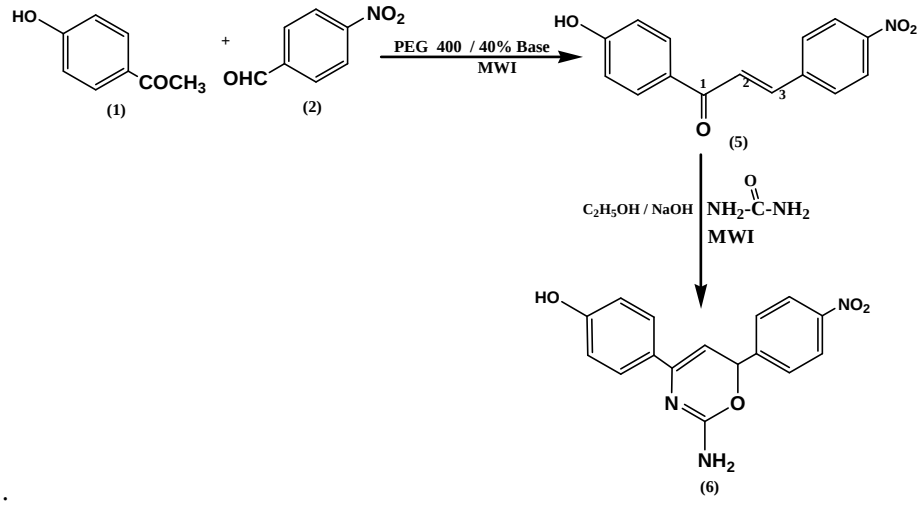
In this work we have synthesized and characterized some of new heterocyclic Derivatives of 4,6-diphenyl substituted oxazine from the chalcones and also studied its microbial activity against gram positive and gram negative bacteria's.

II. Experimental

a. Methods and Materials

The chemicals 4-hydroxyacetophenone **1,** 4-nitrobenzaledehyde **2,** PEG-400 **3**, urea **4**, sodium hydroxide were obtained from Avra chemicals, Hyderabad and were used as such without further purification. Silica gel (TLC and Column grade) were purchased from Merck. The solvents were purified as per the standard procedure reported elsewhere. FTIR spectra (KBr pellets) were measured using Alpha Bruker FTIR instrument scanning with the entire region of 4000 - 400 cm^{-1} with typical resolution of 4.0 cm^{-1}. UV spectra were also recorder using Alpha Bruker UV spectrophotometer. The NMR spectra of the compounds have been recorded on Bruker AV400 spectrometer operating at 400 MHz for recording 1H spectra in DMSO solvent using TMS as internal standard. Mass spectra have been recorded on SHIMADZU spectrometer using chemical ionization technique. Melting points of all synthesised compounds have been determined in open glass capillaries on Mettler FP51 melting point apparatus and are uncorrected. Microwave reaction are carried out commercially available IFB domestic microwave oven having a maximum power output of 110W operating at2450Hz

Scheme - 1 Synthesis of subsituted 4,6-diphenyloxazine



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b. Preparation of 1-(4-hydroxyphenyl)-4-(4-nitrophenyl)but-3-en-1-one (5)

i) Conventional Method:

A mixture of 4-hydroxyacetophenone (0.01mol) and 4-nitrobenzaldehyde (0.01mol) and NaOH (0.02 mol) were stirred in PEG-400 (20mL) as solvent at 60^{o}C for 1 hour. The completion of the reaction was monitored by TLC and the crude mixture was worked up in ice-cold water (100 mL). The product was separated out and filtered. The filtrate was evaporated to dryness to remove water leaving behind PEG-400. The recovered PEG-400 has been utilized for the synthesis of chalcones. Synthesized compounds were recrystallized from ethanol to afford pure compound (**5**). (Yield – 40 % & melting point: 101-102^{o}C).

ii) Microwave irradiation method

A mixture compounds **1**(0.01mol) and **2** (0.01mol) and NaOH (0.02mol) were grinded in to the mortar. Then it was mixed with 20mL of PEG – 400. The mixed compounds were taken in a 100mL beaker and it was irradiated in a microwave oven for the 3 minutes at 110 W operating at 2450Hz at 30 seconds of intervals. After completion of reaction as followed by T.L.C examination, chilled water was added to the reaction mixture and neutralized by an acid. The solid product was obtained, which was filtered, dried and crystallized from an ethanol. The filtrate was evaporated to dryness to remove water leaving behind PEG-400. (Yield – 90% & melting point: 101-102^{o}C)

c. Preparation of 4-(2-amino-6-(4-nitrophenyl)-6H-1,3-oxazin-4-yl)phenol (6)

i) Conventional method:

A mixture of compound 5 (0.01mol), urea (0.01 mol) were dissolved in ethanolic sodium hydroxide (5ml) was stirred about 2-3 hours with a magnetic stirrer. This was then poured into 200 ml of cold water with continuous stirring for an hour and then kept in refrigerator for 24 hours. The crude mixture was poured into ice cold water and neutralized by an acid. The precipitate obtained was filtered, washed and recrystallized. The completion of the reaction was monitored by TLC. The solid product was obtained. (Yield: 60% & melting point: 132-133^{o}C)

ii) Microwave method:

A mixture of compound 5 (0.01mol), urea (0.01 mol) and sodium hydroxide (0.005mol) were mixed thoroughly in mortar. Then it was dissolved into minimum amount of ethanol. The mixed compounds were taken in a 100mL beaker and it was irradiated in a microwave oven for the 5 minutes at 110 W operating at 2450Hz at 30 seconds of intervals. The completion of the reaction was monitored by TLC and the crude mixture was worked up in ice-cold water (100 mL). Acidified with Dil.HCl, the product was separated out and filtered. (Yield: 85% & 132-133^{o}C).

III. Results and Discussion

Spectral details of 1-(4-hydroxyphenyl)-4-(4-nitrophenyl)but-3-en-1-one (5)

UV (λmax: nm): 227 ($\pi \rightarrow \pi^*$ transition), 269 ($n \rightarrow \pi^*$ transition).

FTIR (cm^{-1}): 3441 (O-H), 3107 (Aromatic C-H str), 2854 (C-H), 1744 (C=O), 605 (C=C str), 1524 (NO_2 str.), 855 (C-H out plane bending),1431(C-N str.) **(Figure – 1).**

1H NMR (ppm): 6.25 - 6.29 (2d, 2H, -C**H**=C**H**-), 6.9-8.01 (m, 8H, Ar-**H**), 11.106 (s, 1H, Ar-O**H**) **(Figure – 2).**

Mass (m/z): Calculated M.W 269.0 Observed M.W 270.1

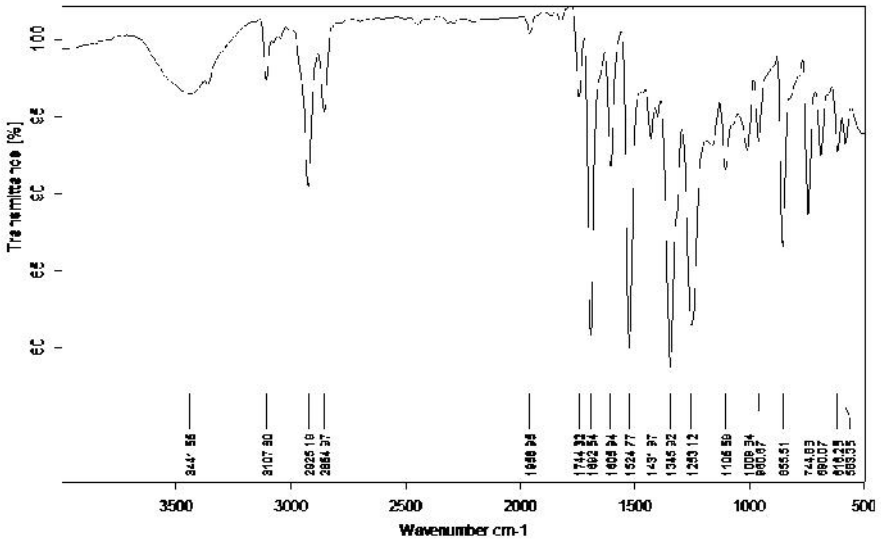

Figure - 1 FTIR spectrum of 1-(4-hydroxyphenyl)-4-(4-nitrophenyl)but-3-en-1-one

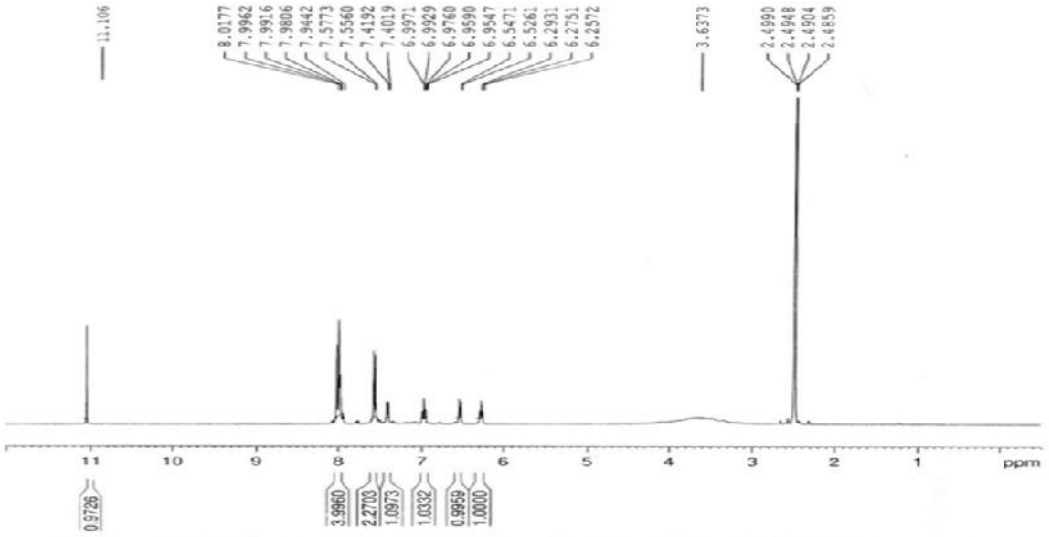

Figure - 2 1HNMR spectrum of 1-(4-hydroxyphenyl)-4-(4-nitrophenyl)but-3-en-1-one

<u>Spectral details of 4-(2-amino-6-(4-nitrophenyl)-6H-1,3-oxazin-4-yl)phenol (6)</u>

UV (λmax: nm): 227 ($\pi \rightarrow \pi^*$ transition), 269 ($n \rightarrow \pi^*$ transition)

FTIR (cm^{-1}): 3377 (O-H), 3107 (Aromatic C-H str), 1689 (NH_2), 1453(Ar-C=C),
1547 (NO_2 str.), 754 (C-H out plane bending), 1431(C-N str.) **(Figure – 3)**

1H NMR (ppm): 2.4(s, 2H,Ar-**NH_2**), 3.3- 3.4(d, H, C=C**H**), 4.7-4.77(d, 1H, C**H**-)
6.6-7.73(m, 8H, Ar-**H**), 9.3(s, 1H, Ar-O**H**

Mass (m/z): Calculated M.W 311.096 , Observed M.W 312.0

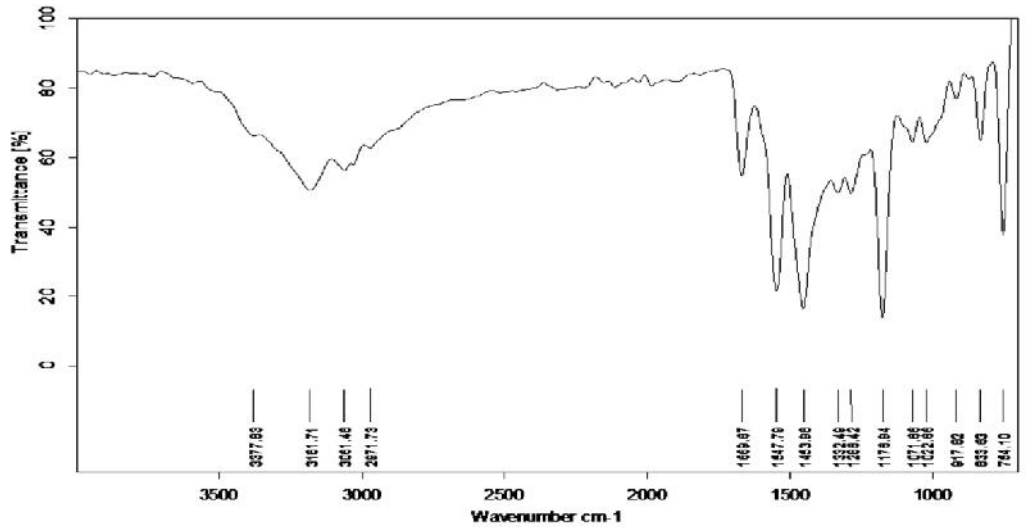

Figure - 3 FTIR spectrum of 4-(2-amino-6-(4-nitrophenyl)-6H-1,3-oxazin-4-yl)phenol

Figure (**1-2**) revealed the FTIR and 1HNMR spectra of 1-(4-hydroxyphenyl)-4-(4-nitrophenyl)but-3-en-1-one (**5**) respectively using compound **1** and **2** with compound **3** in the presence of sodium hydroxide has been shown in the scheme 1. Figure (3-4) revealed the FTIRs and 1HNMR spectra of 4-(2-amino-6-(4-nitrophenyl)-6H-1,3-oxazin-4-yl)phenol (**6**)respectively using compound **5** with compound **4** in the presence of NaOH as catalyst has also been presented in the scheme 1.

UV absorption and FTIR spectra of compound **5** has been provided a preliminary idea in confirmation the formation of product. According to the UV spectrum, presence of peaks at 227 and 269nm clearly showed that the compound (5) has -CH=CH- group and hetero atom respectively. According to the FTIR, represented in Figure (1), presence of peak at 1434 cm^{-1} has clearly noticed the utilization of starting materials transforms into the product. Further, the corresponding peaks at 3441, 3107, 2854, 1744 and 1524 cm-1 have been related to –OH, C-H aromatic stretching and aliphatic C-H stretching, carbonyl and C-N stretching respectively in the compound **5**. The concerned mass of compound **5** is in good agreement with the observed (269.0m/z) and calculated value (270.0 m/z). Similarly, proton NMR strongly empowered for the formation of the product by its δ value at 11.106, 6.9-8.01, and 6.25-6.29 ppm corresponding to the O-H, Ar-H and –CH=CH- protons of compound 5 were mentioned in **Figure (2).**

UV absorption and FTIR spectra of compound **6** has provided a preliminary idea in confirmation the formation of product. According to the UV spectrum of compound **5**, presence of peaks at 227 and 269 nm has been related to aromatic double bond and hetero atom respectively. According to the FTIR, represented in Figure (**3**), absence of peak at 1744 cm^{-1} clearly observed the complete utilization of starting materials transformed into the product. Further, the corresponding peaks at 3377, 3107, 1689 and 754 cm-1 for –OH, C-H are aromatic stretching, NH_2 stretching and C-H bending vibrations respectively in the compound **6**. All such stretching and bending peaks have also been supported for the formation of the product. The concern mass of compound **6** are in good agreement with the observed (312.07 m/z) and calculated value (311.096 m/z). Similarly, proton NMR strongly empowered for the formation of the product by its δ value at 9.3, 6.6-7.73, 4.7 -4.77, 3.33-3.4, 2.4 and 2.48-3.42 ppm corresponding to the O-H, Ar-H, C-H, C=C-H and NH_2 protons of compound **6** .

IV. Antimicrobial activity

The minimum inhibitory concentration (MIC), which is considered as the least concentration of the sample which inhibits the visible growth of a microbe was determined by the broth dilution method. The compounds 5 and 6 were adopted for broth dilution method to evaluate the MIC values. The MIC values are given in the following table.

Table – 1: Sample Minimum Inhibiting Concentration **(MIC)** (µg/ml)

Compounds	***Satphylococcus aureus (S. a)***	***Escherichia coli (E. c)***
5	**62.5**	**62.5**
6	31.25	31.25

Conclusions

- In the present work 4,6-diphenyl substituted oxazines derivatives were prepared successfully by Claisen-Schmidt condensation using green synthetic method. Use of PEG-400 as solvent, which is non-toxic, eco-friendly, water soluble and potentially recyclable with maximum yield. (Stage I & II). While comparing the conventional and microwave reactions, later has excellent than the former in overall aspects.

- The chemical structures of compounds **5** and **6** have been confirmed using various spectral techniques viz., FTIR, UV-Visible, Mass and 1H-NMR spectra and were found to have good agreement with the chemical structures as expected.

- The microbial activities substituted chalcones and 4,6-diphenyl substituted oxazine derivatives were checked against the two microbes ***Staphylococcus aureus*** and ***Escherichia coli.*** The report of antimicrobial activity clearly showed that, the synthesized compound of **6** has moderate activities towards the tested bacterial strains of both gram positive and gram negative than the compound **5**.

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