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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF OXAZINE DERIVATIVES OF 3, 4 – SUBSTITUTED CHALCONE.

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ABSTRACT

The importance of chalcones and oxazines is highly potential and potent in chemistry and biology. Therefore we Synthesized oxazines derivatives of 3, 4- substituted chalcone [substituted -3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one] using urea in alcohol solution of NaOH in good yields. All synthesized compounds were characterized by spectral techniques. Further, the compounds were carried out for anti-bacterial and anti –fungal activities

Keywords: Chalcone, oxazines, sodium hydroxide, ethanol, Claisen -Schmidt condensation, anti-bacterial and anti-fungal activities

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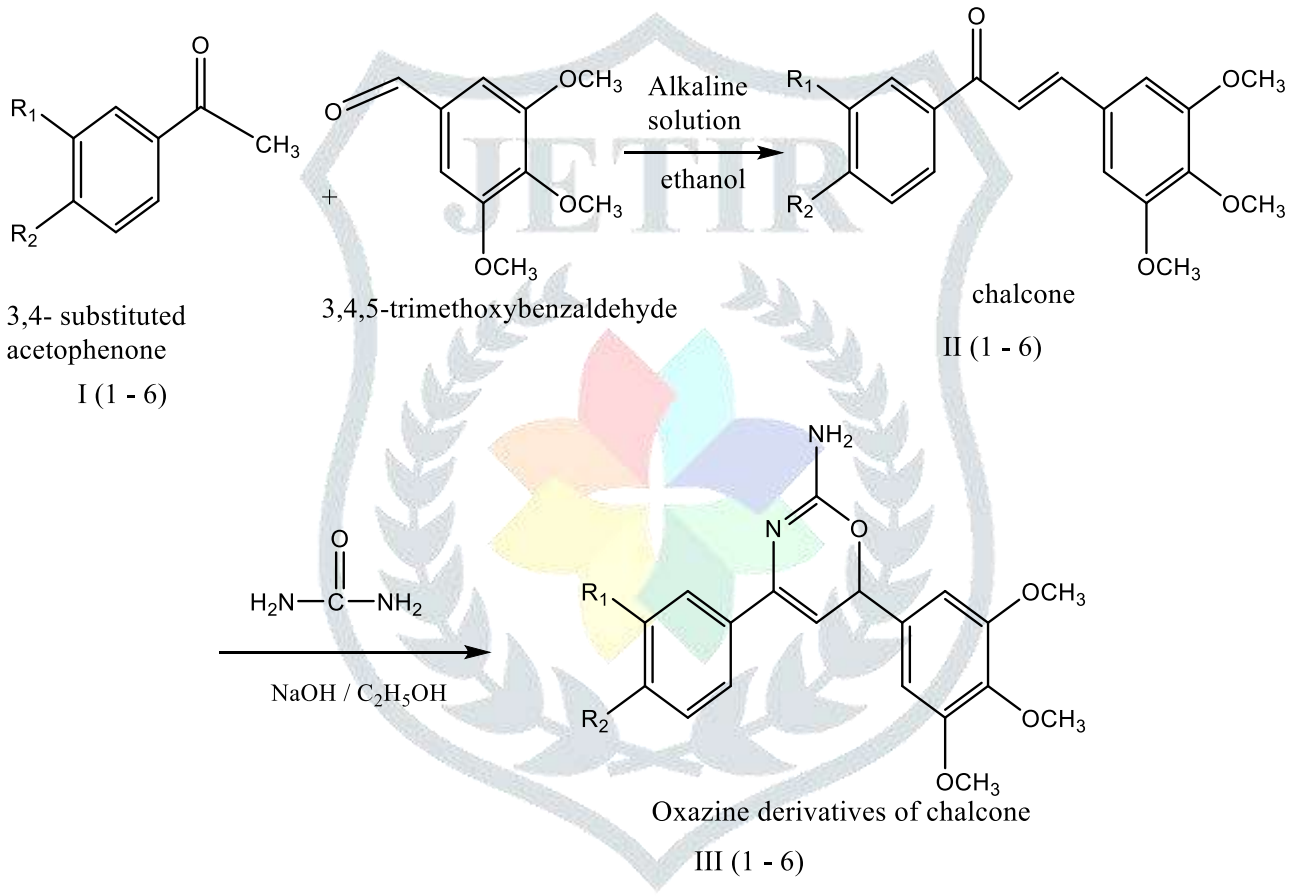
Introduction: Chalcone is an α, β-unsaturated ketone having the $C_6H_5COCH=CHC_6H_5$ structural moiety. It contains two benzene rings with different substituent's and a enone like alkene which increase the reactivity [1-4]. Hence modifications in chalcone structure required to reduce its toxicity and enhance biological activity. Besides of this, the oxazines is a six-membered ring with one oxygen and one nitrogen atom in a cyclohexa-1,3-diene ring. The relative position of the heteroatoms and double bonds determine the isomers of oxazines. Oxazines have many biological activities and are used in a variety of applications[5-6], including antimicrobial, antifungal, anti-inflammatory, anticonvulsant, antitumor, antihypertensive, antipyretics, antioxidant, etc.

Therefore the medicinally active and less cytotoxic new oxygen and nitrogen containing heterocyclic intermediates of chalcone have been synthesized. The modification of the chalcone structure might enhance the biological activity with favorable solubility and reduced toxicity [7-10].Some synthesized analogs of chalcone showed better antibacterial antifungal activity. The structures of the synthesized new chalcone-oxazine compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data. They have been carried out biological activities.

SCHEME



3,4- substituted acetophenone

I (1 - 6)

3,4,5-trimethoxybenzaldehyde

chalcone

II (1 - 6)

Oxazine derivatives of chalcone

III (1 - 6)

Compound	R_1	R_2
I-III(1)	F	OCH_3
I-III(2)	Cl	CH_3
I-III(3)	Cl	OCH_3
I-III(4)	OH	OH
I-III(5)	NH_2	H
I-III(6)	NO_2	H

Materials and methods.

All the reagents and solvents used are of analytical grade purchased from Sigma Aldrich, Merk and Avro. The molecules are extracted in organic solvents, were dried using anhydrous sodium or magnisium sulphate. Melting points were taken in open capillary tubes and are uncorrected. Thin layer chromatography was performed using E. Merck aluminium supported precoated silica gel plates (60F-254). Silica gel of mesh size 60–120 and reagent grade solvents were used for the column chromatography. Thin layer chromatography samples were visualized by UV detector.

IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers. ^{1}H- NMR (400 MHz) and ^{13}C -NMR (100 MHz) spectra were recorded using dimethyl sulphoxide containing tetra methyl silane (TMS) as internal reference was recorded on Bruker spectrometer. Chemical shift values were recorded in ppm (δ scale) relative to tetramethyl silane (0.0 ppm).

Analytical analyses were performed on a PerkinElmer 2400. Mass spectra were obtained by Water-Q-TOF Ultima spectrometer. All the prepared compounds were purified either by recrystallization or by column chromatography using silica gel (60 -120 mesh, Merck) as an adsorbent.

General procedure for the synthesis of 4- substituted -6-(3, 4, 5- trimethoxyphenyl) 6H-1, 3-oxazine - 2 –amine III (1 -6): A mixture of substituted acetophenone **I(1-6)** 0.02 mol and 3, 4, 5-trimethoxybenzaldehyde 0.02 mol was stirred using magnetic stirrer at room temperature in an ethanolic solution of potassium hydroxide for 2-3 hours. The formed yellowish crystals were filtered off washed with distilled water, dried and recrystallized from ethanol to give the chalcone **II(1-6)**[11-12] by using Claisen –schmidt condensation reaction**.**

The substituted -4-(3, 4, 5-trimethoxyphenyl) 6H-1, 3-oxazine -2 –amine **III(1 - 6)** were prepared by stirring the mixture of substituted -3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one **II (1-6)** (0.02 mol), urea (0.02 mol) and ethanolic solution of NaOH in a round bottomed flask using magnetic stirrer for 4hrs. The completion of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured in ice cold water and the product was filtered and recrystallized from ethanol. The compounds subjected to characterization[13-15].

6-(3-fluoro-4-methoxyphenyl)-4-(3, 4, 5-trimethoxyphenyl)-6H-1, 3-oxazin-2-amine, III (1):

This compound was prepared from (E)-1-(3-fluoro-4-methoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (7.16g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: Yellow solid. **M.P:** 109-110 ^{o}C. **Yield:** 78.5% (6.05g)

IR (KBr): 3162–2953 cm^{-1} (Ar-CH), 1675 cm^{-1} (C=O), 1549 cm^{-1} (C=C); 1394 cm^{-1} (OCH_3), 3510-3460 cm^{-1} (Ar-NH_2)

^{1}H-NMR ($CDCl_3$) δ (ppm): 3.71-3.83 (bs, 12H, OCH_3), 5.56 (bs, H, CH of oxazine ring) and 6.52 – 6.97 (m, 5H, Ar-CH), 6.81 (s, 2H, oxazine ring- NH_2)

^{13}C-NMR ($CDCl_3$) δ (ppm): 56.1, 60.8, 72.2, 103.8, 109.1, 112.6, 117.2, 121.4, 131.1, 134.9, 138.4, 142.3, 148.7, 150.0, 153.0, and 154.0

Mass (*m/z*): 388.14

Anal. Calcd. (%). For $C_{20}H_{21}FN_2O_5$: C, 61.85; H, 5.45; F, 4.89; N, 7.21; O, 20.60. Found: C, 61.89; H, 5.47; F, 4.90; N, 7.17; O, 20.57.

6-(3-chloro-4-methylphenyl)-4-(3, 4, 5-trimethoxyphenyl)-6H-1, 3-oxazin-2-amine, III (2):

This compound was obtained from (E)-1-(3-Chloro 4-methylphenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (6.52g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: Pale yellow solid. **M. P:** 117-118 ^oC **Yield:** 73.5% (5.24g)

IR (KBr): 3165–2960 cm^{-1} (Ar-CH), 1670 cm^{-1} (C=O), 1556 cm^{-1} (C=C); 1390 cm^{-1} (OCH_3); 3505-3450 cm^{-1} (Ar-NH_2)

^{1}H-NMR ($CDCl_3$) δ (ppm): 2.23 - 2.29 (d, 6H, CH_3); 3.71-3.92 (bs, 9H, OCH_3), 5.56 (s, H, CH of oxazine ring), 6.52 – 7.14 (m, 6H, Ar-CH), 6.81 (s, 2H, NH_2)

^{13}C-NMR ($CDCl_3$) δ (ppm): 18.8, 19.1, 56.1, 60.8, 72.2, 103.8, 117.2, 121.1, 128.9, 130.4, 131.1, 135.7, 137.0, 138.5, 142.3, 153.0, and 154.0.

Mass (*m/z*): 388.12

Anal. Calcd. (%). For $C_{21}H_{24}N_2O_4$: C, 61.78; H, 5.44; Cl, 9.12; N, 7.20; O, 16.46. Found: C, 61.82; H, 5.47; Cl, 9.13; N, 7.17; O, 16.43.

6-(3-chloro- 4-methoxylphenyl)-4-(3, 4, 5-trimethoxyphenyl)-6H-1, 3-oxazin-2-amine, III (3):

It was prepared from (E)-1-(3-chloro 4-methoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (7.26g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: White solid. **M.P:** 121-123^{o}C. **Yield:** 67.5% (5.45g)

IR (KBr): 3155–2940 cm^{-1} (Ar-CH), 1660 cm^{-1} (C=O), 1549 cm^{-1} (C=C), 1385 cm^{-1} (OCH_3), 3500-3456 cm^{-1} (Ar-NH_2)

^{1}H-NMR ($CDCl_3$) δ (ppm): 3.71-3.88 (bs, 12H, OCH_3), 5.56 (s, H, CH of oxazine ring), 6.52 – 7.46 (m, 6H, Ar-CH), 6.81 (s, 2H, NH_2)

^{13}C-NMR ($CDCl_3$) δ (ppm): 55.3, 56.1, 60.8, 71.4, 103.8, 111.8, 117.2, 122.8, 127.2, 127.9, 131.1, 135.3, 138.4, 142.3, 153.0, 153.5, and 154.0

Mass (*m/z*): 404.11

Anal. Calcd. (%). For $C_{20}H_{21}ClN_2O_5$: C, 59.34; H, 5.23; Cl, 8.76; N, 6.92; O, 19.76. Found: C, 59.31; H, 5.20; Cl, 8.78; N, 6.94; O, 19.77.

4-[2-amino-4-(3, 4, 5-trimethoxyphenyl)-*6H*-1, 3-oxazin-6-yl] benzene-1, 2-diol, III (4):

This compound was obtained from (E)-1-(3, 4-dihydroxyphenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (6.6g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: Yellow solid. **M. P:** 143-145°C. **Yield:** 71.7% (5.33g)

IR (KBr): 3155–2940 cm^{-1} (Ar-CH), 1660 cm^{-1} (C=O), 1549 cm^{-1} (C=C); 1385 cm^{-1} (OCH_3); 3500-3456 cm^{-1} (Ar-NH_2), 3610 -3650 cm^{-1} (Ar-OH)

^{1}H-NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 6.77 (s, H, CH of oxazine ring) 6.52 – 6.67 (m, 5H, Ar-CH), 6.81 (s, 2H, NH_2), 9.48 (bs, 2H, OH)

^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 72.2, 103.8, 114.2, 115.2, 117.2, 122.1, 131.1, 135.6, 138.4, 142.3, 145.2, 145.5, 153.0, 154.0

Mass (*m/z*): 372.13

Anal. Calcd. (%). For $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52; O, 25.78. Found: C, 61.25; H, 5.38; N, 7.55; O, 25.82.

5-(3-aminophenyl)-3-(3, 4, 5-trimethoxyphenyl)-*6H*-1, 3-oxazin-2-amine III (5):

This compound was synthesized from (E)-1-(3-aminophenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (6.26g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: Orange solid. **M.P:** 135-139°C. **Yield:** 65.9% (4.67g)

IR (KBr): 3155–2940 cm^{-1} (Ar-CH), 1660 cm^{-1} (C=O), 1549 cm^{-1} (C=C); 1385 cm^{-1} (OCH_3); 3500-3456 cm^{-1} (Ar-NH_2)

^{1}H-NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 5.28 (s, 2H, Ar-NH_2), 5.56 (s, H, CH of oxazine ring), 6.52 – 7.12 (m, 7H, Ar-CH), 6.81 (s, 2H, oxazine ring- NH_2)

^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 71.9, 103.8, 113.9, 114.1, 117.1, 117.2, 129.7, 131.1, 138.4, 142.3, 148.6, 153.0, 154.0

Mass (*m/z*): 355.15

Anal. Calcd. (%). For $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82; O, 18.01. Found: C, 64.21; H, 5.96; N, 11.82; O, 18.01

5-(3-nitrophenyl)-3-(3, 4, 5-trimethoxyphenyl)-*6H*-1, 3-oxazin-2-amine III (6):

It was prepared from (E)-1-(3-nitrophenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one (6.86g, 0.02 mol), urea (1.2g, 0.02 mol) and 25 cm^3 ethanolic solution of NaOH (0.8g, 0.02 mol).

Colour: Pale yellow solid. **M. P:** 149-151°C. **Yield:** 68.1% (5.24g)

IR (KBr): 3155–2940cm^{-1} (Ar-CH), 1660 cm^{-1} (C=O), 1549 cm^{-1} (C=C); 1385 cm^{-1} (OCH_3); 3500-3456 cm^{-1} (Ar-NH_2), 1310-1400 cm^{-1} (Ar-NO_2)

^{1}H-NMR ($CDCl_3$), δ (ppm): 3.71-3.83 cm^{-1} (bs, 9H, OCH_3), 5.56 cm^{-1} (s, H, CH of oxazine ring), 6.52 – 8.22 cm^{-1} (m, 7H, Ar-CH), 6.81 cm^{-1} (s, 2H, oxazine ring- NH_2)

^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 70.9, 103.8, 117.2, 122.8, 123.5, 129.8, 131.1, 133.2, 138.4, 142.3, 142.5, 148.1, 153.0, 154.0

Mass (*m/z*): 385.13.

Anal. Calcd. (%). For $C_{19}H_{19}N_3O_6$: C, 59.22, H, 4.97; N, 10.90; O, 24.91. Found: C, 59.18, H, 4.95; N, 10.93; O, 24.94.

Anti-microbial activity: The compounds associated with anti-bacterial and antifungal activity are the drugs that destroy microbes and prevent their multiplication or growth. Antimicrobial susceptibility testing methods are divided into two types based on the principle applied in each system. They include disc diffusion (Stokes method and Kirby-Bauer method) method and dilution (Broth dilution and Agar dilution) method. The most commonly employed method is disc diffusion method.

Paper discs impregnated with the test substances were placed on the surface of the Muller Hinton Agar medium inoculated with the target organisms. The plates were incubated and the zones of inhibition around each disc were measured[16].

The synthesized compounds **III (1-6)** were carried out for antibacterial and anti-fungal activity by disc diffusion method against Gram-positive bacteria (*Bacilus subtilius,Streptococcus*), Gram-negative bacteria (*Escherichia coli*, *Proteus*) and against fungus *C. albican*.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number

of standard test procedures. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.[17-18]

Materials required:

- Petriplates
- Nutrient agar medium
- DMF
- Sterile discs

Procedure:

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria [*Bacilus subtilius(BS), Streptococcus aureus (SA*)] and Gram-negative bacteria[*Escherichia coli(EC), Proteus(P*)] in DMF by disc diffusion method on nutrient agar medium and also Anti-fungal activity of the synthesized compounds was determined against *Trichoderma harzianum(TH), Aspergillus niger(AN), Colletotrichum capsici(CC), Aspergillus tamari(AT), Aspergillus flavus(AF), Alternaria solani(AS), and Penicillium oxalicum(PO)*.

The sterile nutrient agar medium (15 cm^3) in each petriplates was uniformly smeared with cultures of Gram-positive, Gram-negative bacteria and fungi. Sterile discs of 10 mm diameter (Hi-Media) was placed in the petriplates, to which different concentrations of drug (20,40,80,100µg/disc) of the synthesized compounds were added.

Ciprofloxacin is used as positive control for comparison of anti-bacterial activity whereas Nystatin is used as positive control for the comparison of anti-fungal activity. For each treatment, three replicates were maintained. The plates were incubated at 37°C for 24 hours and the zone of inhibition was determined**.**

Table: Antimicrobial activity of the synthesized compounds III(1-6)

Compounds	Inhibition zone in mm								
	SA	EC	TH	AN	*CC*	*AT*	*AF*	*AS*	*PO*
III(1)	19	25	9	15	11	16	**18**	15	17
III(2)	**23**	27	13	18	13	21	**21**	19	12
III(3)	**25**	**32**	15	13	15	17	**24**	**21**	16
III(4)	**22**	28	10	**19**	17	22	**22**	**23**	**22**
III(5)	20	**29**	12	**20**	12	19	11	**21**	**21**

III(6)	**24**	23	14	17	14	21	**24**	12	18
Ciprofloxacin	22	28							
Nystatin			17	19	18	23	13	20	21

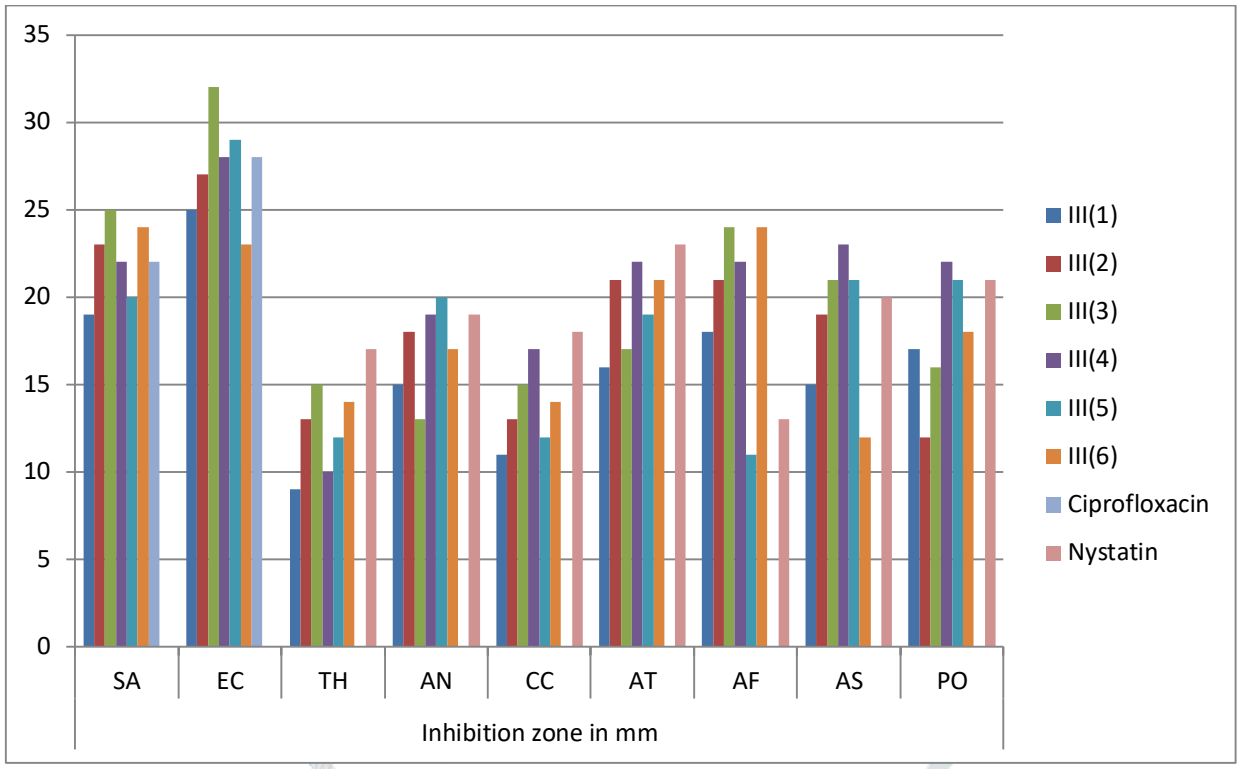
Result and discussion: Substituted II(1-6) were prepared in good yields by Claisen-Smidth condensation reaction of substituted acetophenone I(1-6) with 3, 4, 5-trimethoxybenzaldehyde in the presence of potassium hydroxide as base and water-ethanol mixture as solvent. Substituted chalcone containing α, β-unsaturated compounds II(1-6) were prepared in good yields.

The substituted -4-(3, 4, 5-trimethoxyphenyl) 6H-1, 3-oxazine -2 –amine **III(1 - 6)** were prepared by stirring the mixture of substituted -3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one **II (1-6)** (0.02 mol), urea (0.02 mol) and ethanolic solution of NaOH in a round bottomed flask using magnetic stirrer for 4hrs. The substituted oxazine derivative of 3,4-substituted chalcone were prepared in good yield.

Resistance to number of anti-microbial agents among a variety of clinically significant bacteria is becoming increasingly important. There are various problems arising with the use of anti-microbial such as local tissue irritation, interference with wound healing process, hypersensitivity reactions, system toxicity, narrow anti-microbial spectrum, & emergency of resistance. So, the increasing clinical importance of drug resistant microbial pathogens has additional urgency in microbiological and anti-fungal research. A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules.

The synthesized compounds were tested in vitro for their antimicrobial activity against 2 Gram-positive & 2 Gram-negative bacteria & a yeast type fungi C. *albican* strains. Commercial antibiotics such as Ciprofloxacin and Nystatin were used as reference drugs. The results were compared with reference drugs and depicted in the above table. The table reveals that compounds **III(b), III(3), III(4)** and **III(5)** active against *S.aureus.* Compounds **III(3**) and **III(5)** active against gram negative bacteria *E. coli***.** and fungus *Trichoderma harzianum***. III(4)** and **III(5)** active against fungus *Aspergillus niger.*The all compounds remians inactive against fungus *Colletotrichum capsici* and *Aspergillus tamari***. All tested compound except III(5) active against** *Aspergillus flavus***.** Compounds **III(3), III(4)** and **III(5)** are active against *Alternaria solani***. III(4) and III(5)** showed active against *Penicillium oxalicum***.** Other compound remains inactive against the particular bacteria and fungi.

Graph: Antimicrobial Inhibition zone of the compounds **III (1 - 6)**



Conclusion: We succeeded in the synthesis of oxazine derivatives of 3,4-substituted chalcone and their microbial activities. Some synthesized compounds were very active against fungi, others were remains inactive compared to the standard nystatin and some of the compounds were active against bacteria compared to the standard Ciprofloxacin which is already available in the market.

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