Synthesis, Characterization and Evaluation of Antimicrobial Properties of Novel Oxazine and Thiazine Compounds Derived from Chalcones

SHREEKANT. M. PRAJAPATI1*, SANJAY. S. SHAH2 and ARUN. R. MALAVIYA3

1*,3Department of Chemistry, Hemchandracharya North University, Patan, Matarvadi Part, Gujarat 384265, India.
2Department of Chemistry, Shri U. P. Arts, Smt. M. G. Panchal Science & V. L. Shah Commerce College, Pilvai, Gujarat, India
*Corresponding author E-mail: shreekant12041991@gmail.com

http://dx.doi.org/10.13005/ojc/390530
(Received: August 03, 2023; Accepted: September 28, 2023)

ABSTRACT

In this ongoing research, our objective is to synthesize oxazines and thiazines by employing chalcones, providing an uncomplicated approach for generating diverse heterocyclic derivatives. These substances show a wide spectrum of biological impact, i.e. analgesic, anti-phlogistic, and anti-convulsant properties. To characterize these newly synthesized compounds, we conducted comprehensive analyses employing techniques such as infrared spectroscopy (IR), proton nuclear magnetic resonance (1H NMR), carbon-13 nuclear magnetic resonance (13C NMR) and mass spectrometry. Additionally, we evaluated the antimicrobial potential of these compounds.

Keywords: Chalcone, Antimicrobial, Oxazine, Thiazine.

INTRODUCTION

Oxazines and Thiazines, two classes of organic compounds, exhibit a diverse array of biological activities, making them subjects of significant interest in the field of chemical and pharmaceutical research. In this comprehensive report, the researcher provides an intricate account of the synthesis of a series of chalcones denoted as 1C1 to 1C4. These chalcones were meticulously crafted through Claisen-Schmidt condensation reactions, wherein a variety of substituted benzaldehydes were skillfully combined with acetophenone in the presence of an alkali catalyst, a well-established methodology as outlined in previous scientific investigations1,2. Following the synthesis process, the chalcones underwent a rigorous purification regimen and a thorough characterization that encompassed both physical and spectral analyses. This multifaceted characterization was crucial in confirming the structural integrity and purity of the chalcones. The next pivotal step in this research involved the transformation of the synthesized chalcones into novel Oxazines3-5 and Thiazines6-8, denoted as 1C1OX to 1C4OX and 1C1TH to 1C4TH, respectively. These transformations were achieved...
through distinct reactions with urea and thiourea, resulting in the formation of compounds with significant pharmacological potential. To elucidate the structures of these newly synthesized compounds, the research team relied on a robust array of analytical techniques, including IR spectroscopy, $^1$H NMR spectroscopy, $^{13}$C NMR spectroscopy, and mass spectrometry. These methods provided invaluable insights into the molecular compositions and arrangements of the Oxazines and Thiazines. Furthermore, the study encompassed antimicrobial screening assays$^9$-$^{10}$ to evaluate the potential bioactivity of these compounds. The screening assays were conducted with the use of DMSO as the solvent, and the results were meticulously recorded. This phase of the research aimed to assess the compounds’ efficacy against various microbial strains, shedding light on their potential as antimicrobial agents. Subsequently, the resulting product underwent a process of recrystallization in ethanol, a crucial step in enhancing the purity and crystalline structure of the synthesized compounds, thereby ensuring their suitability for further studies and potential applications. This research represents a significant contribution to the field, offering a comprehensive exploration of the synthesis, characterization, and potential bioactivity of these newly developed Oxazines and Thiazines.

**MATERIAL AND METHODS**

In our experimental procedures, we employed analytical-grade chemicals exclusively. The determination of uncorrected melting points was conducted using the open capillary technique, and the assessment of purity was executed via thin-layer chromatography (TLC). For spectroscopic analysis, Fourier-transform infrared (FTIR) spectra and proton nuclear magnetic resonance ($^1$H NMR) spectra were acquired utilizing a Varian 400MHz spectrometer, with CDCl$_3$ serving as the solvent and tetramethylsilane (TMS) as the reference standard. Elemental analysis was carried out using a Thermofinigan Flash EA instrument (Italy), and the sulfur and halogen content was calculated using the carious method. To evaluate antibacterial and antifungal activity, our research team conducted Broth Dilution method tests against a diverse spectrum of both Gram-positive and Gram-negative bacteria, as well as fungi, as detailed in Table 1.

**Synthesis of 1-(3-fluoro-4-hydroxyphenyl)ethanone (A)**

In carbon di sulphide, a mixture of 2 fluoro phenol (0.1 mole) and Acetyl chloride (0.1 mole) reflux for 30 min in presence of aluminium chloride and it was allowed to stay for around 30 min until the hydrochloride gas solution stopped. The carbon di sulphide was then distilled out. Thereafter, the whole Product was steam distilled. The nonvolatile product was 3 fluoro 4 hydroxy Acetophenone.

**Synthesis of 1-[4-(benzyloxy)-3-fluorophenyl]ethanone(BFE)(1)**

In a 100 mL flask, we combined 1-(3-fluoro-4-hydroxyphenyl)ethanone (0.1 mol), (bromo methyl) benzene (0.1 mol), and Di pottasium carbonate (0.1 mol) with acetone as the solvent. The reaction mixture was vigorously agitated for 7 h under reflux conditions at a degree varying from 50°C to 60°C. After the reaction mixture had cooled to room temperature, 100 mL of cold water was added to quench it. The resulting 1-[4-(benzyloxy)-3-fluorophenyl]ethanone was then filtered and thoroughly rinsed.

**Synthesis of 1-(4-(benzyloxy)-3-florophenyl)-4-phenyl)-3-(sub phenyl) prop-2-en-1-one(1C1to 1C4)**

**General Procedure**

A solution was prepared by dissolving 1-[4-(benzyloxy)-3-fluorophenyl]ethanone (0.01 mol) and replaced aromatic aldehydes (0.01 mol) in 30 milliliters of C$_2$H$_5$OH. In this solution, a 10% caustic soda solution was steadily added. TLC was used to track the reaction's development after the mixture was agitated for 4 hours. Subsequently, the mixture was added into 400 milliliters of icy water were mixed consistently and then counterbalanced using a 1:9 hydrochloric acid and water solution. The reaction mixture was left to stay as it is for a night in a freezer. The resulting expedite was filtered, cleaned, and subjected to recrystallization via ethanol.

**Synthesis of Oxazines and Thiazines Via Chalcones**

A mixture containing 0.01 mol of 1-(4-(benzyloxy)-3-fluorophenyl)-4-phenyl)-3-(sub phenyl) prop-2-en-1-one, 1.0 g of potassium hydroxide (KOH), and 0.01 mol of either Urea or Thiourea was prepared and dissolved in 30 mL of ethanol. This mixture was refluxed in a water bath at a temperature range of 70-80°C for 3 h, with continuous monitoring using thin-layer
chromatography (TLC). Afterward, the solid product that formed was left undisturbed overnight and then purified through recrystallization using ethanol. Notably, the interaction between chalcone and urea led to the production of Oxazine, while the reaction with thiourea produced Thiazine.

### Table 1: Result of Antimicrobial activity of oxazine and thiazine derivatives

<table>
<thead>
<tr>
<th>No</th>
<th>Coding No</th>
<th>Concentration on minimal inhibition µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli MTCC 443</td>
<td>Staphylococcus aureus MTCC 96</td>
</tr>
<tr>
<td>1</td>
<td>1C1OX</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>1C2OX</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>1C3OX</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
<td>1C4OX</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>1C1TH</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>1C2TH</td>
<td>62.5</td>
</tr>
<tr>
<td>7</td>
<td>1C3TH</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>1C4TH</td>
<td>50</td>
</tr>
</tbody>
</table>

Stan CHLORAMPHENICOL 50 50
dard CIPROFLOXACIN 25 50
drug GRESEOFULVIN 500

FT-IR(KBr cm⁻¹): 2930 (C-H Str.Vib) 3047
(Aromatic C-H) 1511,1455 (C=C strVib), 1081 C-O-C str. vib, 1620 (-C=O str.vib), 811 (C-Fstr.vib)

RESULTS AND DISCUSSION

**Characterization of BFE and chalconedervatives**

1-[4-(benzylkoxy)-3-fluorophenyl]ethanone (BFE) (1)

**Product** - 66.20%, m.p.- 103°C-105°C, LC-MS:

**Reaction Scheme**

Fig. 1. Reaction Scheme
1H NMR (400 MHz, CDCl₃): 5.314 (s, 2H, O-CH₂), 2.539 (s, 3H, COCH₃), 7.0-7.7 (m, 8H, Aromatic)

13C NMR (400 MHz, CDCl₃): 26.50 (1C, -COCH₃), 71.30 (1C, O-CH₂), 110-175 (12C, Aeromatic), 196.07 (1C, -CO-) Theoretical for C₁₅H₁₃FO₂: C-73.36, H-5.36 Obtained: C-73.33, H-5.34

1-[4-(benzyloxy)-3-fluorophenyl]-4-phenylbut-2-en-1-one (1C₁)
Product: 61.33%, m.p.-111-113°C, LC-MS: m/z 332.37
FT-IR (KBr cm⁻¹): 3060 (Aromatic C-H), 1593, 1494 (C=C strVib), 1043 (C-O-C str.vib), 1654 (-C=O str.vib), 982 (CH=CH bending, 761(C-F str.vib)
1H NMR (400 MHz, CDCl₃): 5.217 (s, 2H, O-CH₂), 7.55-8.0 (m, 2H, CH=CH), 7.0-7.8 (m, 13H, Aromatic).

13C NMR: 71.11 (1C, O-CH₂), 110-175 (18C, Aeromatic), 163.52 (1C, -CO-), 100-150 (2C, CH=CH). Theoretical for C₂₂H₁₇FO₂: C-79.50, H-5.16 Obtained: C-79.48, H-5.15

(1-(4-(benzyloxy)-3-fluorophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (1C₂)
Product: 60.37%, m.p.-141-143°C, LC-MS: m/z 366.82
FT-IR (KBr cm⁻¹): 3028 (Aromatic C-H), 1567, 1517 (C=C strVib), 1655 (-C=O str.vib), 982 (CH=CH bending, 787(C-F str.vib)
1H NMR (400 MHz, CDCl₃): 5.214 (s, 2H, O-CH₂), 7.55-8.0 (m, 2H, CH=CH), 7.0-7.8 (m, 12H, Aromatic).

13C NMR: 71.09 (1C, O-CH₂), 110-175 (18C, Aeromatic), 187.758 (1C, -CO_), 100-150 (2C, CH=CH) Theoretical for C₂₃H₁₆ClFO₂: C-72.04, C-72.02 Obtained: C-72.02, H-5.42

1-[4-(benzyloxy)-3-fluorophenyl]-4-(4-hydroxyphenyl) but-2-en-1-one (1C₃)
Product: 58.22%, m.p.-160-162°C, LC-MS: m/z 348.37

Characterisation of Synthesized Oxazines
Synthesis of 4-(4-(benzyloxy)-3-fluorophenyl)-6-phenyl-6H-1,3-oxazin-2-amine (1-C₁ OX)
Product: 55.33%, m.p.-256-258°C, LC-MS: m/z 374 FT-IR (KBr cm⁻¹): C-O-C (symmetric) 1230, C-O-C (asymmetric) 1036, NH₂ (amine) (N-H starching) 3435, N-H (bending) 1596, C-N stretching 1337
1H NMR (400 MHz, CDCl₃): 2.544 (s, 2H, -NH₂), 5.219 (s, 2H, -O-CH₂), 7.55-8.0 (m, 2H, CH=CH), 7.0-7.8 (m, 12H, Aromatic).

13C NMR: 71.09 (1C, O-CH₂), 110-175 (18C, Aeromatic), 163.52 (1C, -CO-), 100-150 (2C, CH=CH). Theoretical for C₂₃H₁₇NO₂: C-76.65, H-5.31, N-3.89 Obtained: C-76.62, H-5.28, N-3.88.
4-(4-(benzyloxy)-3-fluorophenyl)-6-(2-chlorophenyl)-6H-1,3-oxazin-2-amine (1-C-2 OX)

**Product:** 61.11%, m.p.: 233-235°C, LC-MS: m/z 409.58

FT-IR (KBr cm⁻¹): C-O-C (symmetric) 1227, C-O-C (asymmetric) 1041, NH₂ (amine) (N-H stretching) 3435, N-H (bending) 1528, C-N stretching 1337.

¹H NMR (400MHz, CDCl₃): 2.543 (s, 2H, NH₂), 5.127-5.220 (s, 2H, -OCH₂), 7.0-7.7 (m, 14H, Aromatic).

¹³C NMR (400MHz, CDCl₃): 71.073 (1C, –OCH₂), 100-160 (18C, Aromatic), 115-125 (1C, C=N), 157.456 (1C, C-NH₂), 115-140 (2C, C=C). Theoretical for C₂₃H₁₈ClFNO₂: C-69.96, H-4.59, N-3.55. Obtained: C-69.93, H-4.56, N-3.58

Characterisation of Synthesized Thiazines

4-(4-(benzyloxy)-3-fluorophenyl)-6-phenyl-6H-1,3-thiazin-2-amine (1-C-1 TH)

**Product:** 63%, m.p.: 211-213°C, LC-MS: m/z 390.2

FT-IR (KBr cm⁻¹): C-O-C (symmetric) 1275, C-O-C (asymmetric) 1018, NH₂ (amine) (N-H starching) 3435, N-H (bending) 1561, C-N stretching 1327.

¹H NMR (400MHz, CDCl₃): 2.547 (s, 2H, NH₂), 5.220 (s, 2H, -OCH₂), 7.0-7.9 (m, 15H Aromatic).

¹³C NMR (400MHz, CDCl₃): 70.834 (1C, –OCH₂), 100-160 (18C, Aromatic), 115-125 (1C, C=N), 157.838 (1C, C-NH₂), 115-140 (2C, C=C). Theoretical for C₂₃H₁₉FN₂O₂S: C-70.75, H-4.86, N-7.17, S-8.21. Obtained: C-70.73, H-4.84, N-7.15, S-8.21

4-(4-(benzyloxy)-3-fluorophenyl)-6-(2-chlorophenyl)-6H-1,3-thiazin-2-amine (1-C-2 TH)

**Product:** 61.90%, m.p.: 244-246°C, LC-MS: m/z 425.2

FT-IR (KBr cm⁻¹): C-O-C (symmetric) 1248, C-O-C (asymmetric) 1025, NH₂ (amine) (N-H starching) 3434, N-H (bending) 1607, C-N stretching 1330.

¹H NMR (400MHz, CDCl₃): 2.543 (s, 2H, NH₂), 5.220 (s, 2H, -OCH₂), 7.0-7.7 (m, 14H Aromatic).

¹³C NMR (400MHz, CDCl₃): 70.737 (1C, –OCH₂), 100-160 (18C, Aromatic), 115-125 (1C, C=N), 157.787 (1C, C-NH₂), 115-140 (2C, C=C). Theoretical for C₂₃H₁₈FN₂O₂S: C-65.01, H-4.27, N-6.59, S-7.55. Obtained: C-65.00, H-4.25, N-6.56

3-(2-amino-4-(4-(benzyloxy)-3-fluorophenyl)-6H-1,3-thiazin-6-y)phenol (1-C-3 TH)

**Product:** 55.33, m.p.: 267-269°C, LC-MS: m/z 406.3.
FT-IR (KBr cm⁻¹): C-O-C (symmetric) 1276, C-O-C (asymmetric) 1079, NH₂ (amine) (N-H starchying) 3435, N-H (bending) 1667, C-N stretching 1276.

¹H NMR (400MHz, CDCl₃): 2.544 (s, 2H, -NH₂), 5.220 (s, 2H, -O-CH₂), 7.0-7.7 (m, 14H Aromatic), 4.0-7.0 (s, 1H, Ar-OH).

¹³C NMR (400MHz, CDCl₃): 71.401 (1C, -OCH₂), 100-160 (18C, Aromatic), 115-125 (1C, -C=), 115-140 (2C, C=C), 157.168 (1C, C-NH₂) Theoretical for C₂₃H₁₉FN₂OS: C-67.96, H-4.71, N-6.89, S-7.89 Obtained: C-67.98, H-4.73, N-6.90, S-7.91.

4-(4-(benzyloxy)-3-fluorophenyl)-6-(4-methoxyphenyl)-6H-1,3-thiazin-2-amine (1-C-4 TH) Product-68.66%, m.p.- 281-283°C, LC-MS: m/z421.4.

FT-IR(KBr cm⁻¹): C-O-C (symmetric) 1247, C-O-C (asymmetric) 1037, NH₂ (amine) (N-H starchying) 3435, N-H (bending) 1583, C-N stretching1269.

⁰H NMR (400MHz, CDCl₃): 2.544 (s, 2H, -NH₂), 5.221 (s, 2H, -O-CH₂), 7.0-7.8 (m, 14H Aromatic), 3.865 (s, 3H, O-CH₃).

⁰¹C NMR (400MHz, CDCl₃): 71.064 (1C, -OCH₂), 100-160 (18C, Aromatic), 115-125 (1C, -C=), 157.890 (1C, C-NH₂) 55.304 (1C, O-CH₃), 115-140 (2C, C=C) Theoretical for C₂₄H₂₁FN₂O₂S: C-68.55, H-5.03, N-4.52, S-7.63. Obtained: C-68.56, H-5.04, N-4.54, S-7.64.

4-(4-(benzyloxy)-3-fluorophenyl)-6-(4-methoxyphenyl)-6H-1,3-thiazin-2-amine (1-C-4 TH) Product-68.66%, m.p.- 281-283°C, LC-MS: m/z421.4.

Antimicrobial activity

We conducted in vitro antimicrobial assessments of the synthesized compounds, namely 1C1OX, 1C2OX, 1C3OX, 1C4OX, 1C1TH, 1C2TH, 1C3TH, and 1C4TH, against bacterial strains S. aureus and E. coli, in addition to the fungal strain C. albicans. Using the Broth Dilution Method, these compounds' antibacterial activity was assessed. Chloramphenicol and Ciprofloxacin served as standard drugs for bacterial strains, while Greseofulvin was used as a reference for fungi.

CONCLUSION

In this article, we have outlined our preliminary endeavors aimed at uncovering novel and potentially active oxazine and thiazine compounds derived from chalcones. Notably, Compound 1C1TH exhibited the most significant inhibitory effect on the growth of Gram-positive S. aureus, with a Minimum Inhibitory Concentration (MIC) value as low as 50 µg/mL. were compound 1C3TH and 1C4TH shows moderate MIC value 62.5 µg/mL. The Gram-negative E.coli bacterial growth is inhibited by compound 1C1TH at A minimum inhibitory concentration (MIC) of 50 µg/mL. were compound 1C3TH, 1C4TH shows moderate MIC value 62.5 µg/mL. Oxazines and Thiazines exhibited potent antifungal activity. Among all the synthesized oxazine and thiazine compounds, specifically 1C1OX, 1C1TH, and 1C4OX, these three compounds demonstrated notable antifungal efficacy. They exhibited strong activity against C. albicans fungi at a concentration of 500 µg/mL, comparable to Griseofulvin¹¹. Newly discovered Oxazines and Thiazines are chemically active components that readily undergo different types of substitutions on their heterocyclic rings, enabling them to display various biological effects. These novel chemical compounds offer medicinal chemists an opportunity to create and advance
Oxazine and Thiazine derivatives as potential lead compounds in drug invention.

ACKNOWLEDGEMENT

I am thankful to my guide Dr. S. S. Shah Sir and Dr. Saddam. S. Sipai for their valuable guidance and is also obliged to the Chemistry Department, Arts, Science & Commerce College Pilvai, Gujarat, India for facilitating with needed facilities for the research work. I am also thankful to the Baroda analytical lab, COE vapi and Spark lab Hyderabad for providing the necessary spectral data.

Conflict of Interest

There are no competing interests are found.

REFERENCES