Synthesis of (2Z)-4,6-Diphenyl-N-((2-(Piperidin-1-yl)Ethyl)-2H-1,3-Thiazin-2-Imino Hydrochloride and its Antimicrobial Activities

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OBO preparation and writing of the manuscript. Authors BJO and APO supervision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aims to synthesize, characterize, and screen (2Z)-4,6-diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-imino hydrochloride for microbial activities.

Methodology: A (2Z)-4,6-diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-imino hydrochloride was synthesized via two-steps reaction from chalcone using acetophenone and benzaldehyde, further cyclized with thiourea and later N-alkylated with 1-(2-chloroethyl)piperidine hydrochloride, its purity was tested by thin-layer chromatography (TLC) and characterized by Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (NMR) and nuclear magnetic resonance (13C-NMR) and screened against Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, methicillin-susceptible Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, and Candida albicans using the standard microbiological method.

Results: A golden yellow needle-like crystals (2Z)-4,6-diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-iminium hydrochloride with Rf 7 EtOAc: 3 Pet (0.75) and MP, 285-287°C was synthesized and the in vitro antimicrobial study of the compound exhibited moderate activities in comparison with standard ciprofloxacin and itraconazole.

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Conclusion: A new (2Z)-4,6-Diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-iminium hydrochloride was synthesized using Claisen-Schmidt condensation, Michael addition and N-alkylation shows moderate antimicrobial activities against E. coli, B. subtilis, methicillin-susceptible S. aureus, methicillin-resistant S. aureus and C. albicans.

Keywords: Chalcone; thiazine; piperidine; Claisen-schmidt; N-alkylation.

1. INTRODUCTION

Heterocyclic compounds are cyclic structures with at least one heteroatom in the ring. Nitrogen, oxygen, and sulfur are the most common heteroatoms. Heterocyclic compounds are essential components of hormones, vitamins, amino acids, and synthesized drugs. They are also useful as copolymers, adhesives, and molecular engineering. They play a vital role in the metabolism of all living organisms. Heterocyclic chemistry is a branch of organic chemistry accounting for more than 90% of new drugs because of the scientific insight discovery and application in chemistry and biology [1]. There are massive numbers of pharmacologically active heterocycle drugs, serving, as a core template for the development of various therapeutic agents.

Chalcones are the core of various important biological and heterocyclic compounds. They are the real intermediate in the synthesis of heterocycle and the unique reagents in organic synthesis. Thiazine is a heterocyclic six-membered compound with four carbon atoms, one nitrogen, one sulfur atom with the configuration of 1,2; 1,3 and 1,4 isomers in respect to the positions of the heteroatoms (Fig. 1). One of the methods of synthesizing 1,3-thiazine is through intramolecular cyclization of thiourea and chalcone (Michael acceptor). The heterocyclic compound of sulfur among the three main heteroatoms that is very useful in the production of many chemicals used in pharmaceuticals, agrochemicals, materials and synthetic intermediates. Thiazine derivatives are beneficial units with diverse biological and showed more potent pharmacological activities as anti-microbial [2-10], anti-inflammatory [11], anti-diabetic [12], anti-convulsant [13-14], anti-tubercular [15], anti-oxidant [16-18] and anti-tumor [19], etc.

The dynamic nature of our population (Nigerian is 200,512,373 as of June 2019 according to United Nations estimates) and resistance to antimicrobial agents (render the existing drugs ineffective, the persistence of infections in the body, increasing the risk of spread, and imposes huge costs on individuals and society. These factors make the existing heterocyclic compounds to be inadequate for pharmaceutical and other industries.

Due to the importance of the heterocyclic compound of sulfur with significant biological activities and industrial values, the study, therefore, aims at the synthesis of 1,3-thiazine with piperidine and screened for microbial activities.

2. MATERIALS AND METHODS

Acetophenone, benzaldehyde, 1-(2-chloroethyl)piperidine hydrochloride, potassium carbonate, sodium hydroxide, acetone, hydrochloric acid and ethanol were purchased from Hopkin and William Ltd, Chadwell Heath, Essex England, UK, BDH Chemicals Ltd, Poole, England UK, Sigma Aldrich and from JHD and were of Analar grade.

Fig. 1. 1,2; 1,3 and 1,4 Isomers of thiazine
Melting points (MP) were measured uncorrected using R000103248 Stuart SMP-10 (Barloworld Scientific Limited apparatus). Fourier-transform infrared spectra (ATR, FT-IR) was recorded using Cary 630 product (Agilent Technologies, USA). $^1$H, $^{13}$C NMR and DEPT 135 (determine the nature of C, CH, CH$_2$) spectra were recorded by Bruker 500 spectrometer with CDCl$_3$ as a solvent. Thin-layer chromatography was performed on 0.25 mm Kieselgel 60, Merck DC pre-coated aluminum plates, and viewed with 254 nm UV lamp.

2.1 Synthesis of Chalcone

A mixture of NaOH (1.10 g, 0.028 mol) in 10 mL water and 5 g of ethanol were added to a 25-mL conical flask and stirred for 15 min in an ice-water bath. An equimolar of freshly distilled acetophenone (2.60 g, 0.022 mol), then benzaldehyde (2.30 g, 0.022 mol) was added at once, stirred at 25-28°C until the thick mixture was formed and kept in the refrigerator overnight. The chalcone was filtered, washed with cold water until neutral to litmus, washed with ethanol, dried, and later recrystallized from ethanol to give pale yellow crystals 3.04 g, 67.49% and MP 56ºC (Scheme 1).

2.2 Synthesis of 4,6-Diphenyl-2H-1,3-Thiazine-2-Imine

Equimolar of 0.01 mol of chalcone (2.08 g) and thiourea (0.76 g) was dissolved in 10-mL ethanolic potassium hydroxide in a 250-mL flask, stirred (300 rpm) at ambient temperature for 3 h. The mixture was poured into cold water, stirred for another 1 h, and kept overnight in the refrigerator. The solid obtained was filtered, triturated with water, dried, and recrystallized from ethanol (2.26 g, 85.2%) and MP 181-183ºC.

2.3 Synthesis of (2Z)-4,6-Diphenyl-N-(2-(Piperidin-1-yl)Ethyl)-2H-1,3-Thiazin-2-Imino Hydrochloride

4,6-Diphenyl-2H-1,3-thiazine-2-imine (2.66 g, 0.010 mol), 1-(2-chloroethyl)piperidine hydrochloride (2.76 g, 0.015 mol), and K$_2$CO$_3$ (3.45 g, 0.025 mol) were mixed in acetone (30 ml) in a 250-mL flat-bottom flask and heated at reflux for 8 h. The cool mixture was poured into ice-water, acidified with 1 M HCl, the semi-solid was collected, and recrystallized from ethanol to give golden yellow needle-like crystals with Rf, 7.7 EtOAc: 3 Pet (0.75) and MP, 285-287ºC (3.12 g, 74.6%).

Scheme 1. Synthesis of N-alkylated 4,6-diphenyl-2H-1,3-thiazine-2-imine
3. ANTIMICROBIAL ACTIVITY

P. aeruginosa, E. coli, K. pneumoniae, B. subtilis, and C. albicans investigated in this study were procured from University of Benin Teaching Hospital while methicillin-susceptible S. aureus, methicillin-resistant S. aureus, and B. subtilis NCTC 8236 gotten from Pharmaceutical Microbiology, University of Benin. All bacterial strains were cultured and subcultured from the stock into sterile nutrient agar, C. albicans on Sabouraud dextrose agar plate at 37°C for 48 h and standardized to 10^6 CFU/mL in 12 h sterile broth before use. Each media were prepared according to the manufacturer's specification. Synthesized N-alkylated 4,6-diphenyl-2H-1,3-thiazine-2-imine (45 mg/mL) was dissolved in dichloromethane and distilled water 1:1 as diluent (solvent control). Ciprofloxacin and itraconazole (30 and 50 mg/mL respectively) were used as the standard for antibacterial and antifungal activities respectively.

3.1 Preliminary Screening (Agar Spot Test)

The sterile molten 25 ml nutrient agar medium was emptied into a 90 mm flat bottom Petri dish, placed on the level surface to ensure uniform thickness of the medium, and dried at 40-50°C for 15 min in hot air oven before usage. A rectangular cavity (rectangle: 4 x 30 mm^2) was bored with a small sterile surgical knife and sealed the base with sufficient warm nutrient agar. The wire loop (2 mm diameter) was used to streak six different standardized inoculums along the cavity and emptied 0.5 ml synthesized 1,3-thiazine into the cavity. The plate was left standing for 1 h at room temperature, incubated at 37°C for 18 h and measured the zones of inhibition diameter. All experiments were carried out in triplicates.

3.2 Determination of Zone of Inhibition Using Agar-well Method

Standardized inoculums of the test microorganisms were radially streaked with an individual cotton swab aseptically on their respective agar plate. A stainless steel sterile borer (8 mm) was used to bore six uniform sizes well, each was uniformly sealed, and filled with 100 µL of four different concentration range of synthesized compound, one for standard and sixth the control (diluent). The plate was left standing for 1 h at room temperature, incubated at 37°C for 18 h and measured the zones of inhibition diameter. All experiments were carried out in triplicates [20].

3.3 Determination of MIC Using Microdilution Broth Method

The minimum inhibitory concentration (MIC) values of synthesized 1,3-thiazine were determined using the microdilution broth method. Four different concentration range of 100 µL of synthesized 1,3-thiazine diluted in double strength sterile Mueller Hinton broth in test tubes, 20 µL of standardized organisms was added and incubated at 37°C overnight. The test compound was the positive control and diluent as the negative control against the microorganisms for the experiment. The MIC recorded as the lowest concentrations without any visible growth (turbidity) for each of the test organisms. All experiments were carried out in triplicates.

4. RESULTS AND DISCUSSION

A new N-alkylated 4,6-diphenyl-2H-1,3-thiazine-2-imine was synthesized and screened for microbial activities. Chalcone was prepared using the Claisen-Schmidt condensation reaction in ethanol sodium hydroxide. 4,6-Diphenyl-2H-1,3-thiazine-2-imine was synthesized from an equimolar mixture of 1,3-diaryl-2-propen-1-one and thiourea at room temperature. 4,6-Diphenyl-2H-1,3-thiazine-2-imine was N-alkylated with 1-(2-chloroethyl)piperidine hydrochloride in good yield 74.6% with one isolable product from TLC. (2Z)-4,6-Diphenyl-N-(2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-imino hydrochloride was assigned structure based on spectroscopic data of FT-IR (cm⁻¹) of the ring 2441, 2404 (C=C), 1238 (C=S), 1096 (C-N), 1238 (C=S), 9248 (CH₂), 3030 (Ar C-H) (Fig. 2) these correlated with book [21] in line with literature [2-3, 22]. ^1H NMR 1.37-1.42 (m, 1H), 1.81-1.85 (d, J_AB =20 Hz, 3H), 2.27-2.37 (q, 2H), 2.67-2.75 (q, 2H), 3.37-3.42 (m, 2H, CH₂), 3.66-3.68 (d, J_AB =10 Hz, 2H), 3.90-3.93 (t, 15 Hz, CH₂), 12.55 (1H, s, HCl) of the piperidine in line with [23] and 7.53 - 7.59, (m, 8H), 7.83 (s, H, C-5) and 8.12-8.15 (m, 2H, Ar) for the thiazine. ^13C NMR (CDCl₃, ppm): 22.08, 22.49, 24.41, 53.50, 56.76, 109.13 (C-5), 127.43 (4C), 127.70, 129.09 (4C), 129.26 (C), 131.54 (2C), 136.14 (C-6), 165.40 (C-4), 170.06 (C-2) (Figs. 4 and 5).
Fig. 2. FT-IR of (2Z)-4,6-diphenyl-N-[(2-(piperidin-1-yl)ethyl]-2H-1,3-thiazin-2-imino hydrochloride

Fig. 3. $^1$H NMR spectra of (2Z)-4,6-diphenyl-N-[(2-(piperidin-1-yl)ethyl]-2H-1,3-thiazin-2-imino hydrochloride

4.1 Antimicrobial Activity

(2Z)-4,6-Diphenyl-N-[(2-(piperidin-1-yl)ethyl]-2H-1,3-thiazin-2-imino hydrochloride was screened against all tested microorganisms using modified agar-well method for the preliminary screening for further studies and had activities except for K. pneumoniae (Table 1). A sensitivity test was carried out to evaluate the zone of inhibition and MIC of N-alkylated-4,6-diphenyl-2H-1,3-thiazine-2-amine using the cup-plate method and microbroth dilution method respectively.
Table 1. Preliminary screening of (2Z)-4,6-diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-imino hydrochloride for antimicrobial activities in mm

<table>
<thead>
<tr>
<th>No</th>
<th>P. aeruginosa</th>
<th>MSSA</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>B. subtilis (T)</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUK 12</td>
<td>18.50 ± 0.95</td>
<td>21.50 ± 0.75</td>
<td>15.50 ± 0.55</td>
<td>N</td>
<td>12.00 ± 0.10</td>
<td>14.50 ± 0.25</td>
</tr>
</tbody>
</table>

Table 2. Zone of inhibition of (2Z)-4,6-diphenyl-N-((2-(piperidin-1-yl)ethyl)-2H-1,3-thiazin-2-imino hydrochloride in mm

<table>
<thead>
<tr>
<th>No</th>
<th>MSSA</th>
<th>E. coli</th>
<th>B. subtilis(T)</th>
<th>B. subtilis</th>
<th>C. albicans</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUK 12</td>
<td>12.00 ± 0.75</td>
<td>11.50 ± 0.35</td>
<td>14.50 ± 0.20</td>
<td>13.00 ± 0.15</td>
<td>11.50 ± 0.35</td>
<td>12.00 ± 0.74</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>31.00 ± 0.33</td>
<td>27.00 ± 0.25</td>
<td>25.00 ± 0.30</td>
<td>28.00 ± 0.20</td>
<td>-</td>
<td>31.00 ± 0.40</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21.50 ± 0.35</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. 4. $^{13}$C NMR spectra of (2Z)-4,6-diphenyl-N-[(2-{piperidin-1-yl})ethyl]-2H-1,3-thiazin-2-imino hydrochloride

Fig. 5. DEPT 135 NMR spectra of (2Z)-4,6-diphenyl-N-[(2-{piperidin-1-yl})ethyl]-2H-1,3-thiazin-2-imino hydrochloride

Table 3. MIC of (2Z)-4,6-diphenyl-N-[(2-{piperidin-1-yl})ethyl]-2H-1,3-thiazin-2-imino hydrochloride in mg/ml

<table>
<thead>
<tr>
<th>No</th>
<th>MSSA</th>
<th>E. coli</th>
<th>B. subtilis(T)</th>
<th>B. subtilis</th>
<th>C. albicans</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUK 12</td>
<td>9.00 ± 0.15</td>
<td>7.50 ± 0.25</td>
<td>7.50 ± 0.10</td>
<td>9.00 ± 0.33</td>
<td>7.50 ± 0.25</td>
<td>9.00 ± 0.25</td>
</tr>
</tbody>
</table>

BUK: Bukky

A zone of inhibition of N-alkylated-4,6-diphenyl-2H-1,3-thiazine-2-imine is presented (Table 2) in comparison with standard ciprofloxacin and itraconazole. BUK 12 inhibits moderately all bacteria and C. albicans except K. pneumoniae. The MIC of BUK 12 has reasonable concentration ranges of 11.50-14.50 mg/ml (Table 3). The evident above reveals that BUK 12 containing piperidine has antimicrobial activities.
5. CONCLUSION

The study presents the synthesis of Chalcone from acetylphenone and benzaldehyde, a two-step sequence of 4,6-diphenyl-2H-1,3-thiazine-2-imine and its N-alkylated with 1-(2-chloroethyl)piperidine hydrochloride. The (2Z)-4,6-diphenyl-N-((2-piperin-1-yl)ethyl]-2H-1,3-thiazin-2-imino hydrochloride was characterized by physically (Rf values, melting point) and spectral data (FT-IR, 1H, 13C-NMR). The in-vitro antimicrobial results reveal that N-alkylated-1,3-thiazine exhibited moderate antibacterial and antifungal activities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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