NEW THIOPHENE BEARING DIMETHYL-5-HYDROXY ISOPHTALATE ESTERS AND THEIR ANTIMICROBIAL ACTIVITIES

Hakan ÜNVER1*, Zerrin CANTÜRK

1Chemistry Department, Faculty of Science, Anadolu University, 26470, Eskişehir, Turkey
2Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

ABSTRACT

Thiophene belongs to a class of heterocyclic compounds. In this study, three new dimethyl-5-hydroxy isophtalate derived thiophene esters were successfully synthesized and characterized by FT-IR, Elemental Analysis, 1H-NMR, 13C-NMR and HR-Mass techniques. Synthesized compounds antimicrobial effects were tested on Staphylococcus aureus (ATCC 25923); Enterococcus faecalis (ATCC 29212); Enterococcus faecalis (ATCC 51922); Klebsiella pneumoniae (ATCC 700603); Pseudomonas aeruginosa (ATCC 27853); Escherichia coli (ATCC 35218); Escherichia coli (ATCC 25922) and Candida albicans (ATCC 90028); Candida glabrata (ATCC 90030); Candida krusei (ATCC 6258); Candida parapsilosis (ATCC 22019). Compound C1 showed the highest antimicrobial activity, possessing the same potential as chloramphenicol against, P. aeruginosa ATCC 27853. According to MTT assays, this compound (C1) was identified as non-toxic.

Keywords: Thiophene, Antibacterial, Anticandidal, MIC, Microbroth dilution, Ketoconazole

1. INTRODUCTION

Thanks to the improving investigations on development of new antibiotics, moderate human life period increasing significantly over time. Discovering new type of bacterial effecting molecules resulted altering several diseases including pneumonia, meningitis, septicemia etc. However, consuming excess amount of antibiotics increasing the bacterial resistance and lead to decreasing the number of bacteria killing molecules day by day [1-4]. Large number of antibiotics have at least one heterocycle like pyridine [5-7], furan [8-10], pyrrole [11, 12], pyrimidine [13, 14] etc. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications. Many thiophene derivatives have been developed as chemotherapeutic agents and are widely used. Thiophene nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities [15-17]. Molecules that have a part of thiophene ring can act antidepressant, anti-inflammatory, anticonvulsant and antiepileptic properties [18]. Also, the number of thiophene ring containing molecules found to be effective against several bacteria are rising and reported in literature day by day [19-22].

In this study, three new thiophene containing dimethyl-5-hydroxyisophtalate esters (Tetramethyl-5,5’-((thiophene-2,5-dicarbonyl)bis(oxy))disophthalate(C1), dimethyl 5-((thiophene-2-carbonyl)oxy) isophtalate(C2), dimethyl 5-(2-(thiophen-2-yl)acetoxy)isophtalate) (C3) was synthesized and characterized with several spectroscopic methods. The antibacterial effect of synthesized molecules are investigated on seven bacteria and four candida with microbroth dilution techniques. The cytotoxic effect of evaluated on healthy mouse fibroblast NIH3T3 cell line.

*Corresponding Author: hakanunver@anadolu.edu.tr
2. MATERIALS AND METHODS

2.1. Chemical

All chemical were used without further purifications. HR-MS measurements were recorded on Schimadzu LCMS-IT-TOF spectrometer. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on Bruker DPX FT spectrometer. FT-IR spectra were recorded with Perkin Elmer Spectrum 100 Spectrometer using KBr discs. Elemental analysis was carried out using Elementar Vario EL III microanalyzer device. Melting points were measured in a Stuart SMP-30 melting point apparatus.

2.2. General Synthesis of Dimethyl-5-hydroxy Isophthalate Derivatives (Compound C₁, C₂ and C₃)

Three new thiophene esters synthesis procedure was depicted in Figure 1.

**Tetramethyl 5,5’-((thiophene-2,5-dicarbonyl)bis(oxy)) diisophthalate (C₁)**

Thiophene-2,5-dicarboxylic acid (0.6g, 0.0029 mol, 1 eq) was suspended in acetonitrile, then the solution was cooled in an ice bath to 0°C. Thionyl chloride (0.63 mL, 0.0087 mol, 3 eq) was added dropwise to the first solution over 30 min. Solution was kept at room temperature for 48 h and excess thionyl chloride and solvent was evaporated under reduced pressure. Oily residue was added dropwise directly to the dimethyl-5-hydroxyisophthalate (0.8 g, 0.0038 mol, 1.3 eq) and triethylamine (NEt₃) (0.6 mL, 0.0043 mol, 1.5 eq) solution in tetrahydrofuran (THF) and the reaction was checked with TLC certain time period. After completion of reaction, excess tetrahydrofuran was evaporated and the residue was poured into cold deionized water. The insoluble product precipitated immediately and filtrated over vacuum filtration, then washed three times with water. Light brown powder obtained (Compound C₁).

Yield: 52%, m.p.:190°C, Anal. Calc. (%) for C₂₆H₂₀O₁₂S: C=56.1; H=3.6; S=5.7; O=34.6. Found: C=55.9; H=3.8; S=5.8; O=34.5. IR (KBr, cm$^{-1}$): 3104.2; 1726.4; 1250.7; 1060.4; 751.6. $^1$H-NMR: (400
MHz, CDCl₃, δ ppm): 8.62 (s, 2H), 8.11 (s, 4H), 8.05 (d, J=3.6 Hz, 2H), 3.96 (s, 12H). ¹³C-NMR: (400 MHz, CDCl₃, δ ppm): 165.20, 159.29, 150.17, 138.51, 134.86, 132.22, 128.57, 127.03, 52.7. MS [M+Na]⁺: m/z 579.05

**Dimethyl 5-((thiophene-2-carbonyl)oxy)isophthalate (C₂)**

Dimethyl-5-hydroxyisophthalate (1.28 g, 0.0061 mol, 1.3 eq) and triethylamine (0.97 mL, 0.007 mol, 1.5 eq) was dissolved in THF then, thiophene-2-carbonyl chloride (0.5 mL, 0.0046 mol, 1 eq) was added dropwise to the first solution at 0 °C. After 24h, excess solvent was evaporated and residue poured into cold water, insoluble product precipitated, filtrated, washed and dried under vacuum. White powder. Yield: 48%, m.p:133 °C, Anal. Calc. (%) for C₁₅H₁₂O₆S: C=56.3; H=3.8; O=29.9; S=10.1. Found: C=56.1; H=3.7; O=30.1; S=10.1. IR (KBr, cm⁻¹): 3106.2; 1728.2; 1250.2; 1064.7; 751.6. ¹H-NMR: (400 MHz, CDCl₃, δ ppm): 8.34 (s, 1H), 7.96 (s, 2H), 7.44 (d, J=5.2 Hz, 1H), 7.06 (s, 1H), 6.99 (t, J=4.4 Hz, 1H), 4.25 (s, 2H), 3.87 (s, 6H). ¹³C-NMR: (400 MHz, CDCl₃, δ ppm): 169.59, 165.05, 151.22, 134.91, 132.09, 128.09, 127.46, 127.41, 127.31, 126.28, 53.20, 34.79. MS [M+Na]⁺: m/z 357.03.

**Cytotoxicity**

Cytotoxicity Cytotoxicity tests were performed using the MTT assay. NIH/3T3 mouse embryonic fibroblast cell line (ATCC® CRL-1658™) obtained from ATCC.

NIH/3T3 cells were incubated in Dulbecco's Modified Eagle's Medium (HyClone, Thermo Scientific, USA) supplemented with fetal calf serum (HyClone, Thermo Scientific, USA), 100 IU/mL penicillin and 100 mg/mL streptomycin (HyClone, Thermo Scientific, USA) at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. NIH/3T3 cells were seeded at 5x10⁴ cells into each well of 96-well plates. After 24 h of incubation, the culture media were removed and compounds were added to culture medium in the range between 3.9 and 500 μM/mL concentrations. Then, cytotoxicity test was performed using the MTT assay, which measures mitochondrial activity, in NIH/3T3 cells. Firstly, 10 μL MTT solution (5 mg/mL MTT powder in PBS) was added and incubated for 3 hours at 37 °C, 5% dimethyl sulfoxide (DMSO) was added. OD values of each well was read at 540 nm. Inhibition % was calculated for each concentration of the compounds according to the formula below [23] and IC₅₀ values were estimated by plotting a dose-response curve of the inhibition % versus concentration [24].

\[
\text{Inhibition} \% = 100 - \left( \frac{\text{OD}_{\text{test compound}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{solvent control}} - \text{OD}_{\text{blank}}} \right) \times 100
\]

**2.2. Antimicrobial Activity**

Antimicrobial activity of the final compounds was evaluated by the broth microdilution method according to the modified NCCLS M27-A2 standard procedure as indicated in the literature [25]. Tested microorganism strains and origins were as follows: *Staphylococcus aureus* (ATCC 25923); *Enterococcus faecalis* (ATCC 29212); *Enterococcus faecalis* (ATCC 51922); *Klebsiella pneumoniae* (ATCC 700603); *Pseudomonas aeruginosa* (ATCC 27853); *Escherichia coli* (ATCC 35218);
Escherichia coli (ATCC 25922); Candida albicans (ATCC 90028); Candida glabrata (ATCC 90030); Candida krusei (ATCC 6258); Candida parapsilosis (ATCC 22019). Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.13 and 1.63 μM/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. Each experiment in the antimicrobial assays was replicated twice in order to define the MIC values. Ketoconazole and chloramphenicol were used as positive control and the results (MIC values) are shown in Table 1.

3. RESULTS AND DISCUSSION

3.1. Characterization of Compounds

The synthesis of stated compounds is given in Figure 1. Compounds C₁, C₂ and C₃ were obtained from the esterification reaction between dimethyl-5-hydroxyisophthalate and thiophene-2,5-dicarboxylic acid (for C₁), thiophene-2-carbonyl chloride (for C₂) and thiophene-2-acetyl chloride (for C₃) respectively. Spectroscopic values of synthesized compounds are given in materials and methods section. (see Supplementary Materials, Figure S1, S12)

In the FT-IR spectra of C₁, C₂ and C₃ compounds, the carbonyl (C=O) group stretching vibration frequency was observed at 1726.4 cm⁻¹, 1728.2 cm⁻¹ and 1731.5 cm⁻¹ respectively. Ester bond C-O vibration peaks was observed between 1250.7 cm⁻¹-1060.4 cm⁻¹ (for C₁), 1250.2 cm⁻¹-1064.7 cm⁻¹ (for C₂) and 1255.6 cm⁻¹-1130.7 cm⁻¹ (for C₃). The C=S bond vibration frequency of thiophene ring was observed at 751.6 cm⁻¹, 751.6 cm⁻¹ and 764.9 cm⁻¹ for C₁, C₂ and C₃ in the same order (see Supplementary Materials, Figure S1-S3).

In the ¹H-NMR spectra of compound 1 (C₁), singlet signal observed at 3.96 ppm is related to methyl (-CH₃) group of phthalate moiety. Same group signal was observed at 3.94 ppm (for C₂) and 3.87 ppm for C₃. Additionally, methylene (-CH₂-) group of C₃ was observed at 4.25 ppm as expected. Aromatic proton peaks of both thiophene and phenyl ring can be seen between 8.62-8.05 ppm for C₁, 8.59-7.18 ppm for C₂ and 8.34-6.99 ppm for compound 3 as it should be. ¹³C-NMR signals of synthesized compounds were in agreement with proposed structures. Aliphatic carbon signals was observed at 52.7 ppm for C₁, 52.6 ppm for C₂ and 53.2 ppm for C₃ compound. Additionally methylene (-CH₂-) group carbon signal was also observed at 34.7 ppm for C₃ (see Supplementary Materials, Figure S4-S9).

Compounds mass spectra are also support the proposed structures. The mass results are found to be, m/z 579.05 for C₁, m/z 343.02 for C₂ and m/z 357.03 for C₃ as expected (see Supplementary Materials, Figure S10-S12).

3.2. Antimicrobial and Cytotoxic Activity

The cytotoxic activities of these compounds were evaluated against a normal mouse embryonic fibroblast cell line, NIH/3T3, for determining the selectivity of potential antimicrobial agents. According to MTT assays, this compound was identified as non-toxic (IC₅₀>500 μM/mL).

The antimicrobial activity was determined by minimal inhibitory concentration (MIC). Compounds (C₁, C₂ and C₃) were screened for their antibacterial and antifungal activity (Table 1). Ketoconazole and chloramphenicol were used as positive control and the results (MIC values) are shown in Table 1. C₂ was effective against P. aeruginosa when compared with chloramphenicol compounds C₁ and C₂ against Staphylococcus aureus, E. faecalis no activity.
Table 1. Antibacterial activity data of compounds tested (μM/mL)

<table>
<thead>
<tr>
<th>Microorganisms/Cell line</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>Chlor-amphenicol</th>
<th>Ketoconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>800</td>
<td>800</td>
<td>400</td>
<td>200</td>
<td>-------</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>-------</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 700603</td>
<td>400</td>
<td>200</td>
<td>200</td>
<td>50</td>
<td>-------</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>800</td>
<td>800</td>
<td>200</td>
<td>50</td>
<td>-------</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 51922</td>
<td>800</td>
<td>800</td>
<td>400</td>
<td>100</td>
<td>-------</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 35218</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>200</td>
<td>-------</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>200</td>
<td>-------</td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 90028</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>-------</td>
<td>200</td>
</tr>
<tr>
<td><em>Candida glabrata</em> ATCC 90030</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>-------</td>
<td>200</td>
</tr>
<tr>
<td><em>Candida krusei</em> ATCC 6258</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>-------</td>
<td>3.125</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em> ATCC 22019</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>-------</td>
<td>200</td>
</tr>
<tr>
<td>NIH/3T3 ATCC®CRL-1658</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>-------</td>
<td>-------</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The successful synthesis of three new thiophene derived dimethyl-5-hydroxyisophthalate esters has been reported. Synthesized compounds were tested for their antibacterial effects and compounds showed significant antibacterial activities. According to the literature, the presence of sulphur atom in a molecule lead to increasing the antibacterial activity [26]. Additionally, the positive charge of donor S atom in heteroaromatic ring lead to increasing the lipophilicity and give higher capability to penetrate the bacteria, so the presence of thiophene ring in the molecule lead to positive increase the antibacterial effect [27]. When compared with chloramphenicol (MIC=200 μM/mL), compounds, were the same effective compound against *Pseudomonas aeruginosa* ATCC 27853 with a MIC value of 200 μM/mL. The other compounds and chloramphenicol showed the same level of activity against *E. coli* ATCC 35218. All compounds were found to be as active as ketoconazole against *Candida parapsilosis* ATCC 22019, whereas showed no significant antifungal activity against *Candida krusei* ATCC 6258. Especially C1 most effective against *Candida albicans* (MIC=50 μM/mL) ATCC 90028. Comparatively, C1 four-fold effect than ketoconazole on *Candida albicans* ATCC 90028. The results indicated that, our findings are in agreement with literature and it can be said that thiophene containing molecules can possess antibacterial effect.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the Anadolu University, Chemistry Department for NMR analysis and Doping and Narcotic Matters Research Laboratory (DOPNA) for HR-Mass Measurements.
REFERENCES


[22] Zhao Q, Li J, Zhang X, Li Z and Tang Y. Cationic Oligo (thiophene ethynylene) with Broad-Spectrum and High Antibacterial Efficiency under White Light and Specific Biocidal Activity against S. aureus in Dark, ACS Appl Mater Interfaces, 2016; 8, 1019–1024.


