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

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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, Elementel Analysis, ^{1}H-NMR, ^{13}C-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 **C_1** showed the highest antimicrobial activity, possessing the same potential as chloramphenicol against, *P. aeruginosa* ATCC 27853. According to MTT assays, this compound (**C_1**) 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))diisophthalate(C_1),dimethyl5-((thiophene-2-carbonyl)oxy) isophthalate(C_2), dimethyl 5-(2-(thiophen-2-yl)acetoxy)isophthalate) (C_3) 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.

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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 C_1, C_2 and C_3)

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

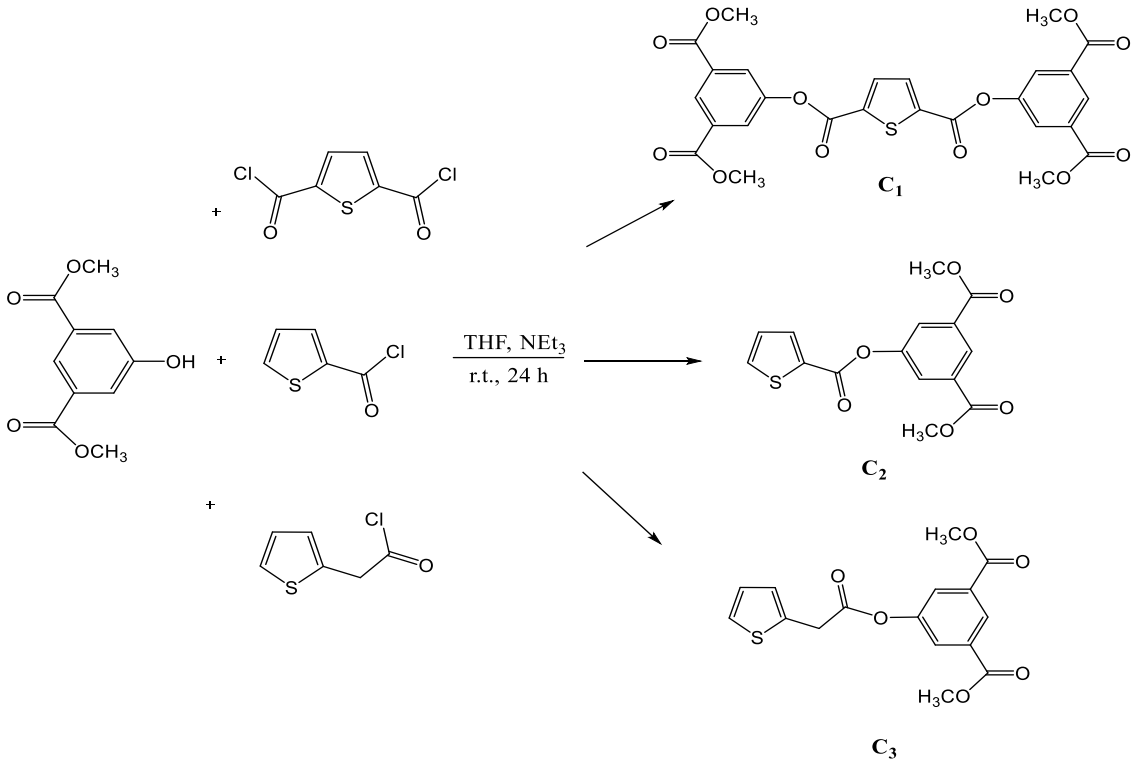

Figure 1: Synthesis procedure of three new dimethyl-5-hydroxy isophthalate esters.

Tetramethyl 5,5'-((thiophene-2,5-dicarbonyl)bis(oxy)) diisophthalate (C_1)

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-hydroxyisophtalate (0.8 g, 0,0038 mol, 1.3 eq) and triethylamine (NEt_3) (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 $C_{26}H_{20}O_{12}S$: 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, $CDCl_3$, δ ppm): 8.62 (s, 2H), 8.11 (s, 4H), 8.05 (d, J=3.6 Hz, 2H), 3.96 (s, 12H). ^{13}C-NMR: (400 MHz, $CDCl_3$, 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 (C_2)

Dimethyl-5-hydroxyisophtalate (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 $C_{15}H_{12}O_6S$: 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. ^{1}H-NMR: (400 MHz, $CDCl_3$, δ ppm): 8.59 (s, 1H), 8.08 (s, 2H), 7.99 (d, J=2.8, Hz, 1H), 7.69 (d, J=4.4 Hz, 1H), 7.18 (t, J=4.4 Hz, 1H), 3.94 (s, 6H). ^{13}C-NMR: (400 MHz, $CDCl_3$, δ ppm): 165.34, 160.08, 150.54, 135.25, 134.19, 132.03, 131.92, 128.22, 128.19, 127.27, 52.60. MS $[M+Na]^+$: *m/z* 343.02.

Dimethyl 5-(2-(thiophen-2-yl)acetoxy)isophthalate (C_3)

Compound C_3 was synthesized according to the same procedure described above. Thiophene-2-acetyl chloride was used instead of thiophene-2-carbonyl chloride. White powder. Yield: 62%, m.p.:51°C, Anal. Calc. (%) for $C_{16}H_{14}O_6S$: C=57.5; H=4.2; O= 28.7; S=9.6. Found: C=57.3; H=4.1; O=29.2; S=9.4. IR (KBr, cm^{-1}): 3048.1; 1731.5; 1433.6; 1255.6; 1130.7; 764.9. ^{1}H-NMR: (400 MHz, $CDCl_3$, 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). ^{13}C-NMR: (400 MHz, $CDCl_3$, 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% CO_2. NIH/3T3 cells were seeded at $5x10^3$ 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].

Inhibition %= $100-[(OD_{\text{test compound}} - OD_{\text{blank}} / OD_{\text{solvent control}} - OD_{\text{blank}})]x100$

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 C_1, C_2 and C_3 were obtained from the esterification reaction between dimethyl-5-hydroxyisophtalate and thiophene-2,5-dicarboxylic acid (for C_1), thiophene-2-carbonyl chloride (for C_2) and thiophene-2-acetyl chloride (for C_3) 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 C_1, C_2 and C_3 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 C_1), 1250.2 cm^{-1}-1064.7 cm^{-1} (for C_2) and 1255.6 cm^{-1}-1130.7 cm^{-1} (for C_3). 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 C_1, C_2 and C_3 in the same order (see Supplementary Materials, Figure S1-S3).

In the ^{1}H-NMR spectra of compound 1 (C_1), singlet signal observed at 3.96 ppm is related to methyl (-CH_3) group of phthalate moiety. Same group signal was observed at 3.94 ppm (for C_2) and 3.87 ppm for C_3. Additionally, methylene (-CH_2-) group of C_3 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 C_1, 8.59-7.18 ppm for C_2 and 8.34-6.99 ppm for compound 3 as it should be. ^{13}C-NMR signals of synthesized compounds were in agreement with proposed structures. Aliphatic carbon signals was observed at 52.7 ppm for C_1, 52.6 ppm for C_2 and 53.2 ppm for C_3 compound. Additionally methylene (-CH_2-) group carbon signal was also observed at 34.7 ppm for C_3 (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 C_1, *m/z* 343.02 for C_2 and *m/z* 357.03 for C_3 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 (IC_{50}>500 μM/mL).

The antimicrobial activity was determined by minimal inhibitory concentration (MIC). Compounds (C_1, C_2 and C_3) were screened for their antibacterial and anticandidal activity (Table 1). Ketoconazole and chloramphenicol were used as positive control and the results (MIC values) are shown in Table 1. C_2 was effective against *P. aeruginosa* when compared with chloramphenicol compounds C_1 and C_2 against *Staphylococcus aureus, E. faecalis* no activity.

Table 1. Antibacterial activity data of compounds tested (μM/mL)

Microorganisms/Cell line	C_1	C_2	C_3	Chlor-amphenicol	Ketoconazole
Staphylococcus aureus ATCC 25923	800	800	400	200	--------
Pseudomonas aeruginosa ATCC 27853	200	200	200	200	--------
Klebsiella pneumoniae ATCC 700603	400	200	200	50	--------
E. faecalis ATCC 29212	800	800	200	50	--------
E. faecalis ATCC 51922	800	800	400	100	--------
E. coli ATCC 35218	400	400	400	200	--------
E. coli ATCC 25922	400	400	400	200	--------
Candida albicans ATCC 90028	50	100	100	--------	200
Candida glabrata ATCC 90030	100	200	100	--------	200
Candida krusei ATCC 6258	100	100	100	--------	3.125
Candida parapsilosis ATCC 22019	200	200	200	--------	200
NIH/3T3 ATCC®CRL-1658	>500	>500	>500	--------	--------

4. CONCLUSION

The successful synthesis of three new thiophene derived dimethyl-5-hydroxyisophtalate esters has been reported. Synthesized compounds were tested for their antibacterial effects and compounds showed significal 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 anticandidal activity against *Candida krusei* ATCC 6258. Especially C_1 most effective against *Candida albicans* (MIC=50 μM/mL) ATCC 90028. Comparatively, C_1 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.

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