



SYNTHESIS AND ANTIBACTERIAL EVALUATION OF 5-*{(E)-[4-(2'',5''-DIOXO-2'',5''-DIHYDRO-1H-PYRROL-1-YL)PHENYL] DIAZENYL-2-METHYL-N³-PHENYL-3-THIOPHENECARBOXAMIDE ANALOGUES*

Habila J.D¹ --- Iyun, O.R.A² --- Ella E.E³ --- Tajuddeen N[†] --- Musa H⁵

^{1,2,4,5} Department of Chemistry, Ahmadu Bello University Zaria, Nigeria

³ Department of Microbiology, Ahmadu Bello University Zaria, Nigeria

ABSTRACT

A series of thiophene-carboxamide analogues (1a-c) were synthesized by incorporating N-phenylmaleimide and 5-amino-4-substituted-(2,4-dimethylphenyl)-2-methyl-3-thiophenecarboxamide and their structures were assigned by studying their spectral data. The synthesised compounds were tested against *Staphylococcus aureus* and *Escherichia coli* using agar-in-well diffusion and broth micro dilution methods. The zone of inhibition values ranged from 20 – 24 mm against the two test organisms for all the compounds tested (1a-c). The result of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) showed that all the test compounds inhibited and completely killed *S. aureus* and *E. coli* at a concentration range of 0.63 – 2.50 µg/mL and .031 – 2.50 µg/mL respectively. The MBC value of compound 1a was the same as that of the standard drug (ciprofloxacin) used on *E. coli* (0.63 mg/mL) and *S. aureus* (0.25 µg/mL).

Keywords: Thiophenecarboxamide derivatives, Antimicrobial activity, Coupling reaction, NMR, Gewald reaction, *E. coli*.

Contribution/ Originality

This study contributes in the existing literature, some new thiophene-carboxamide derivatives and their antibacterial activities.

1. INTRODUCTION

Substituted thiophenes and their biheterocycle derivatives have received considerable attention in the last two decades, due to their wide ranging therapeutic properties [1]. They are used as antimicrobial agents [2] as analgesic [3] as anti-inflammation agents [4] as antioxidant

[†] Corresponding author

agents [5] as antitumor agents [6] and as anesthetic agents [7]. Thiophene molecules, when fused with other heterocyclic rings have given rise to newer compounds with enhanced biological activities.

Diabetic condition and HIV epidemic have led to an increase in the number of immune-compromised patients, which in turn has led to increase in systemic bacterial infections; these in addition to the emergence of drug and multiple drug resistant bacterial strains have become a global concern and have placed a demand for the synthesis of new class of anti-bacterial agent.

Staphylococcus aureus is one of the major human pathogens associated with hospitals and community-acquired infections and has been implicated in many incidences of food poisoning and water contamination [8]. It has also been implicated in Persistent cases of osteomyelitis [9, 10] pulmonary infection in cystic fibrosis [11] and wound abscess [12]. *Staphylococcus aureus* was the initial pathogen that has become resistant to all known antibiotics and has posed a threat for many years [13].

E. coli, in addition to its important role in causing inflammatory and hemorrhagic diarrheal illnesses, is the major cause of neonatal meningitis [14] nosocomial bacteremia and surgical site infections and is the leading cause of urinary tract infections [15]. It is against this background that we investigate the potential of our target molecules to act as antibacterial agents against *S. aureus* and *E. coli*.

Hence, the current research is aimed at exploring the potential of these new classes of thiophene based compounds, as lead candidate in the search for new antibiotic drugs.

Our synthetic approach involve a nucleophilic addition of substituted aromatic amine, to maleic anhydride followed by condensation in the presence of acetic anhydride and sodium acetate to give the desired N-phenylmaleimide. This was coupled to the substituted aminothiophenes, which was prepared by the Knoevenagel-Cope condensation of a carbonyl compound with activated nitrile group, to yield an α,β -unsaturated nitrile. This on reacting with elemental sulfur followed by ring closure gave the desired substituted 2-aminothiophenes [16].

To the best of our knowledge, no biological studies of these classes of compounds have been reported. Hence we present here the syntheses of a new class of thiophene-carboxamide analogues with different electron donating and withdrawing groups (CN, CO₂CH₃, CH₃) and their potential to act as antibacterial agents.

2. MATERIALS AND METHODS

All reagents were of analytical grade, purchased from Sigma-Aldrich (Germany). Melting points were uncorrected and determined using Reichart Austria micro melting point apparatus. Analytical thin layer chromatography (TLC) was performed using Aluminum pre coated Silica gel 60 (F₂₅₄) from Merck (Germany). Visualization was achieved by UV light (254 nm) and spraying with 20 % H₂SO₄ in methanol. NMR spectra were recorded on a Bruker Avance FT NMR spectrometer at 400 MHz for ¹H NMR and at 100 MHz for ¹³C NMR, with residual solvent peak as internal standard. Infra-red (IR) spectra of the solid samples were recorded on a Perkin-Elmer 100SP ATR FT-IR spectrophotometer. The gas chromatography mass spectrometry (GC-MS)

was performed on Agilent 6890 Series, GC system with Agilent 5973 network mass selector. Ultraviolet visible (UV/VIS) analysis was carried out on a Perkin Elmer Lambda 35 UV/VIS spectrometer.

2.1. Chemistry Synthesis of 5-amino-4-substituted-N²-(2,4-dimethylphenyl)-2-methyl-3-thiophenecarboxamide (1, 2)

Ethylcyanoacetate (11.9 g, 0.1 mol) or Malononitrile (3.37 g, 0.1 mol) with N-(2,4-dimethylphenyl-3-oxobutyramide (20.53 g, 0.1 mol), Sulphur (3.39 g, 0.1 mol) and morpholine (9.0 g, 0.1 mol) in a 250 mL round bottom flask, were refluxed in 50 mL of ethanol for 2 hrs at 55-65 °C. The resulting thick dark solution was cooled to room temperature and stored overnight in a refrigerator. After which it was filtered and washed with a small quantity of ethanol and ethanol/water mixture and then dried. The white amorphous powder obtained was recrystallized from ethanol to give a white crystalline solid [17].

2.2. Synthesis of N-phenylmaleamic Acids

To a stirred solution of maleic anhydride (9.8 g, 0.05 mol) in dichloromethane (60 mL) was added a solution of N-phenylaniline (17.32 g, 0.05 mol) at 15-20 °C. The reaction mixture was stirred overnight. Afterward the precipitate formed was filtered, washed and air dried. The crude N-phenylmaleamic acid was recrystallized in dimethylformamide (DMF).

2.3. Synthesis of N-phenylmaleimide Derivative (4, 5)

N-phenylmaleamic acid (0.69 g) was heated for 1hr at 90-100 °C in acetic anhydride (5 mL) in the presence of anhydrous sodium acetate (0.05 g). Afterward the reaction mixture was poured into iced water and stirred continuously for 1 hr. The crystalline product obtained was filtered off and recrystallized in DMF.

2.4. Preparation of Diazonium Solution

Dry sodium nitrite (1.38 g, 0.02 mol) was slowly added over a period of 15 minutes, with stirring to sulphuric acid (10 mL) at 30 °C and then heated to 60 °C for 15 minutes. The solution was then cooled to 5 °C and 20 mL of acetic acid-propionic acid (17:3) was added drop wise with stirring, allowing the temperature to rise to 15 °C. The reaction mixture was then cooled to 0-5 °C, and finely ground aminothiophene (0.02 mol) derivatives were slowly added within 30 minutes period and the temperature maintained below 5 °C. The resulting solution was stirred at 0-5 °C for 2-4 hrs. Excess nitrous acid was decomposed with appropriate amount of urea. The clear diazonium salt solution was used immediately in the coupling reaction.

2.5. Coupling to N-Phenylmaleimide Derivatives (1a-c)

The N-Phenylmaleimide derivatives (0.02 mol) were slowly dissolved in acetic acid (20 mL) and cooled in an ice bath to 0 °C. The diazonium solution previously prepared was added drop wise over a period of 30 minutes, with vigorous stirring (Scheme 3). The mixture was stirred for

an additional 2 hours at 0-5 °C, then sodium acetate solution (10 %) was added slowly drop wise until the pH was between 4 and 5. The product was filtered and washed with warm water, and then with cold water until it was acid-free, then it was air dried. Recrystallization from DMF, yielded the desired product, the purity of the product was checked by thin layer chromatography using ethylacetate and hexane (1:4) as the solvent system.

5-amino-N³-(4-chlorophenyl)-4-cyano-2-methyl-3-thiophenecarboxamide (1)

Brownish grey powder; yield 68%; m pt. 233-235 °C, IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3458, 3315, 3199 (NH), 2206 (CN), 1675 (CO); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 2.36$ (s, 3H, CH₃), 7.36 (d, 2H, J = 2.08 Hz, Ar-H), 7.63 (d, 2H, J = 1.09 Hz, Ar-H), 7.77 (brs, 2H, NH₂), 9.67 (s, 1H, NH); ¹³C NMR (DMSO-d₆): $\delta_{\text{ppm}} = 14.98$ (CH₃), 115.34 (CN), 121.81, 128.41 (CH aromatic), 127.03, 137.86, (aromatic C), 87.99, 112.56, 140.88, 160.43 (thiophene C), 165.23 (CO amide).

Methyl 2-amino-4-[(4-chloroanilino) carbonyl]-5-methyl-3-thiophenecarboxylate (2)

White powder; yield 77%; m pt. 165-167 °C, IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3475, 3330, 3211 (NH), 1664, 1623 (CO); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 2.52$ (s, 3H, CH₃), 3.76 (s, 3H, OCH₃), 7.38 (d, 2H, J = 2.00 Hz, Ar-H), 7.66 (d, 2H, J = 1.96 Hz, Ar-H), 7.78 (brs, 2H, NH₂), 9.80 (s, 1H, NH); ¹³C NMR (DMSO-d₆): $\delta_{\text{ppm}} = 16.36$ (CH₃), 50.70 (OCH₃), 121.57, 128.41 (CH aromatic), 126.86, 138.05 (aromatic C); 105.36, 112.34, 141.02, 161.38 (thiophene C), 165.07 (CO ester), 165.11 (CO amide).

5-amino-4-cyano-N³-(2,4-dimethylphenyl)-2-methyl-3-thiophenecarboxamide (3)

Cream powder; yield 57%; m pt. 234-236 °C, IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3361, 3316, 3207 (NH), 2204 (CN), 1628 (CO); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 2.14$ (s, 3H, CH₃), 2.26 (s, 3H, CH₃), 2.39 (s, 3H, CH₃) 6.97 (s, 1H, Ar-H), 7.04 (d, 1H, J = 7.8 Hz, Ar-H), 7.21 (d, 1H, J = 7.8 Hz, Ar-H), 7.68 (brs, 2H, NH₂), 8.97 (s, 1H, NH); ¹³C NMR (DMSO-d₆): $\delta_{\text{ppm}} = 14.76$, 17.87, 20.48 (CH₃), 115.34 (CN), 125.67, 126.26, 130.61 (CH aromatic), 132.72, 133.66 (aromatic C), 88.25, 133.19, 139.83, 160.51 (thiophene C), 164.97 (CO amide).

Methyl 2-amino-4-[(2,4-dimethylphenyl)anilino]carbonyl]-5-methyl-3-thiophenecarboxylate (4)

Yellow solid; yield 44%; m pt. 128-130 °C, IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3461, 3423, 3318 (NH), 1665, 1634 (CO); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 2.15$ -2.53 (s, 9H, 3CH₃), 3.74 (s, 3H, OCH₃), 6.96 (d, 1H, J = 8.2 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 7.22 (d, 1H, J = 7.8 Hz, Ar-H), 7.71 (brs, 2H, NH₂), 9.04 (s, 1H, NH); ¹³C NMR (DMSO-d₆): $\delta_{\text{ppm}} = 14.38$, 17.91, 20.87 (CH₃), 51.12 (OCH₃), 123.07, 127.36, 131.17 (CH aromatic), 133.24, 133.83, 134.85 (aromatic C), 108.24, 114.22, 141.18, 161.35 (thiophene C), 164.50 (CO ester), 165.98 (CO amide).

N-phenylmaleimide (5)

Yellow solid; yield 77%; m pt. 90-92 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3271, 3207, 3071, 3037 (), 2238, 1876 (CN), 1695 (CO); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 6.84$ (s, 2H, olefinic H), 7.23-7.43 (Ar-H), ¹³C

NMR (DMSO-d₆): $\delta_{\text{ppm}} = 129.16, 127.99, 126.09$ (CH aromatic), 131.22 (aromatic C), 134.22 (CH olefinic), 169.52 (CO amide).

2-nitro-N-phenylmaleimide (6)

White crystals; yield 67.5%; m pt. 130-131 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3485, 3168, 3112 (), 1774, 1708 (); ¹H NMR (DMSO-d₆): $\delta_{\text{ppm}} = 6.94$ (s, 2H, olefinic H), 7.78-8.24 (Ar- H), ¹³C NMR (DMSO-d₆): $\delta_{\text{ppm}} = 125.03, 125.89, 128.90, 135.25$ (CH aromatic), 133.96, 145.31 (aromatic C), 134.66, 134.96 (CH olefinic), 168.45 (CO amide).

4-cyano-N³-(2',4'-dimethylphenyl)-5-[(Z)-[4'''-(2'',5''-dioxo-2'',5''-dihydro-1H-pyrrol-yl)-3''-methoxyphenyl]diazenyl]-2-methyl-3-thiophenecarboxamide (1a)

136.2 (C2), 135.2 (C3 & C4), 142.5 (C5), 135.6 (C1'), 131.1 (C2'), 129.6 (C3'), 133.1 (C4'), 130.9 (C5'), 126.1 (C6'), 159.4 (C7'), 169.8 (C2''& C5''), 132.6 (C3''& C4''), 139.3 (C1'''), 113.3 (C2'''), 126.6 (C3'''&C4'''), 142.5 (C5'''), 112.8 (C6'''), 14.4 (-CH₃, C2), 55.6 (-OCH₃, C5'''), 17.8 (-CH₃, C2'), 20.5 (-CH₃, C4'), 118.7 (-CN, C4)

Methyl 4-[(2',4'-dimethylanilino)carbonyl]-2-[(Z)-[4'''-(2'',5''-dioxo-2'',5''-dihydro-1H-pyrrol-yl)-3''-nitrophenyl]diazenyl]-5-methyl-3-thiophenecarboxylate (1b)

134.9 (C2), 133.2 (C3 & C4), 134.9 (C5), 134.9 (C1'), 130.5 (C2'), 126.1 (C3'), 134.9 (C4'), 130.3 (C5'), 119.8 (C6'), 155.3 (C7'), 169.9 (C2''& C5''-CO₂CH₃, C4), 130.9 (C3''& C4''), 134.9 (C1'''& C4'''), 126.6 (C2'''), 120.5 (C3'''), 155.3 (C5'''), 112.3 (C6'''), 15.1 (-CH₃, C2), 55.7 (-CO₂CH₃, C4), 17.9 (-CH₃, C2'), 20.5 (-CH₃, C4').

Methyl 4-[(2',4'-dimethylanilino)carbonyl]-2-[(Z)-[4'''-(2'',5''-dioxo-2'',5''-dihydro-1H-pyrrol-yl)-3''-methoxyphenyl]diazenyl]-5-methyl-3-thiophenecarboxylate (1c)

134.9(C2), 133.2(C3 & C4), 134.9(C5), 134.9(C1'), 130.5(C2'), 126.1(C3'), 134.9(C4'), 130.3(C5'), 119.8(C6'), 155.3(C7'), 169.9(C2'' & C5''-CO₂CH₃), 130.9(C3'' & 4''), 134.9(C1''' & C4'''), 126.6(C2'''), 120.5(C3'''), 155.3(C5'''), 112.3(C6'''), 15.1(-CH₃, C2), 52.3(-CO₂CH₃, C4), 17.9(-CH₃, C2'), 20.5(-CH₃, C4'), 55.7(-OCH₃).

2.6. Antimicrobial Studies

The antimicrobial activities of the synthesized compounds (1a-c) were determined using microbial strains obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria (ABUTH); Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria; *Escherichia coli*. The cork and bore diffusion method of Karou, et al. [18] was used to determine the antibacterial activity of the test compounds. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24h at 38 °C. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 McFarland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter cork borer was used to

make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the test compounds (1a-c). The plates were incubated for 24h at 38 °C. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibition zone (mm) produced by the test compounds.

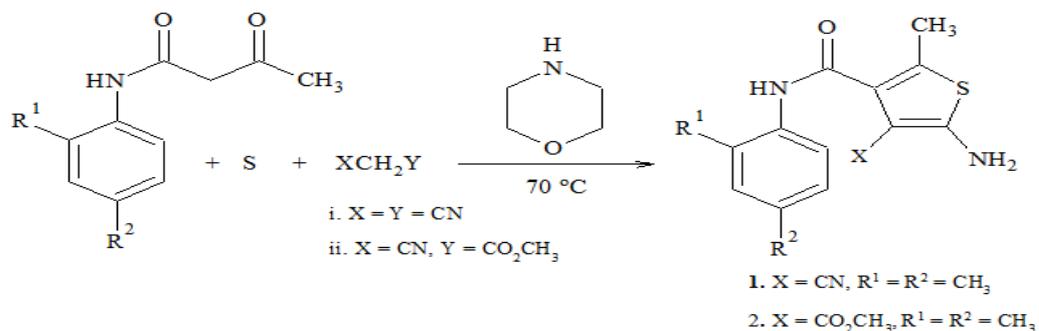
2.7. Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentrations (MIC) were determined for the compounds using micro broth dilution method in accordance with. Serial dilution of the least concentration of the compound that showed activity were prepared using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 38 °C for 18h. Minimum inhibition Concentrations (MIC) was recorded as the lowest concentrations of the compounds showing no visible growth (turbidity) in the broth.

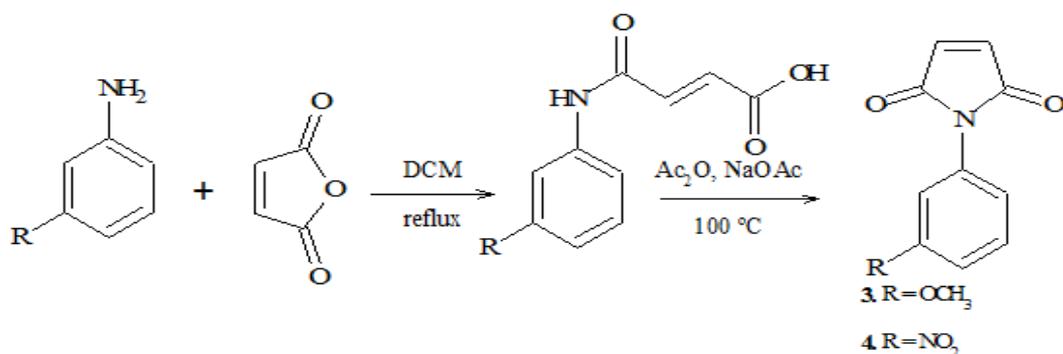
2.8. Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration were determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar (OXOID) plates and incubated at 38°C for 48h. The MBCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

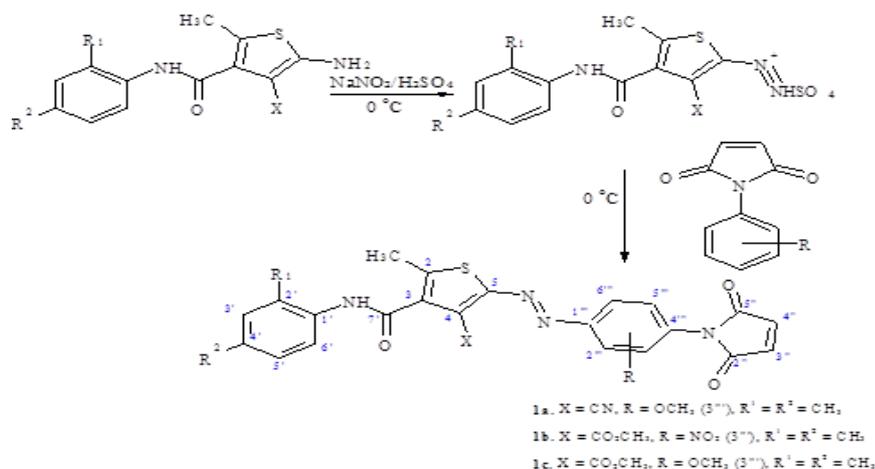
3. RESULTS AND DISCUSSION



Schem-1. Synthesis of amino-thiophenes 1,2,3 and 4



Schem-2. Synthesis of N-phenylmaleimide



Scheme-3. Synthesis of thiophene azo compounds

3.1. Chemistry

The synthetic approach used in this work involved three stages (Scheme 1, 2 and 3). The first step of the reaction is likely the Knoevenagel-Cope condensation of the carbonyl compound, with the activated nitrile (XCH_2Y ; $X=Y=CN$; $X=CN$, $Y=CO_2CH_3$) yielding α , β -unsaturated nitrile. This intermediate was then thiolated at the methylene group of the carbonyl compound by the electrophilic elemental sulfur, followed by ring closure in catalytic amount of morpholine to afford the 2-aminothiophene (intermediates, 1 and 2). The second step (Scheme 2) occurs via a nucleophilic addition of substituted aromatic amine, to maleic anhydride. The reaction takes place in dichloromethane under reflux condition, where the aromatic amine attacks the carbonyl carbon of maleic anhydride, which breaks open forming amide bond and carboxylic acid. This was followed by condensation at 100 °C in the presence of acetic anhydride and sodium acetate to give the desired N-phenylmaleimide (intermediates, 3 and 4). The final step (Scheme 3) involved treatment of 1-2 in nitrosylsulfuric acid ($NaHSO_4$) and acetic anhydride/propionic acid mixture to afford the thiophene diazonium salts which coupled successfully with 3 and 4 in acetic acid containing sodium acetate to give the desired thiophenecarboxamide products (1a-c). The data obtained from analytical as well as spectral studies were consistent with the structures of aminothiophene (1 and 2) and N-phenylmaleimide (3 and 4) (Experimental section). Common features of the aminothiophene derivatives are their strong IR stretching vibration around 1630 cm^{-1} due to amide carbonyl group. The ^{13}C NMR signals at δ 87.99, 112.56, 140.88 and 160.43 confirmed the presence of the thiophene ring.

3.2. Biological Studies

The result of determination of zone of inhibition of all the test compounds (Table 1) ranges from 20–24 mm against the test organisms. Compounds 1a and 1c was observed to be the most active with zone of inhibition of 24 mm against *E. coli* and *S. aureus* respectively, while 1b showed a zone of 22 and 23 mm against *S. aureus* and *E. coli* respectively. The result of the minimum

inhibition concentration (MIC) and minimum bactericidal concentration (MBC) shown in Table 1 showed that all the test compounds (1a-c) inhibited and completely kill *Staphylococcus aureus* at various concentration; Compound 1a (MIC = 0.63 µg/mL, MBC = 1.25 µg/mL), compound 1b (MIC = 1.25 µg/mL, MBC = 2.50 µg/mL) and compound 1c (MIC = 2.50 µg/mL, MBC= 5.00 µg/mL). The MIC of the test compounds on *E. coli* is; Compound 1a (MIC= 0.31 µg/mL, MBC = 0.63 µg/mL), Compound 1b (MIC=1.25 µg/mL, MBC= 1.25 µg/mL) and Compound 1c (MIC= 2.50 µg/mL, MBC= 2.50 µg/mL). When compared with the standard drug ciprofloxacin used as positive control, compound 1a showed the same MBC value of 0.625 µg/mL against *E. coli* and 1.25 µg/mL against *S. aureus*. These results showed that the test compounds possess potential that can be explored in the search for antibacterial drugs.

Table-1. Antimicrobial evaluation of synthesized compounds

Compounds	Zones of inhibition 10 µgmL ⁻¹		MIC µgmL ⁻¹		MBC µgmL ⁻¹	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
1a	22.0	23.5	0.625	0.31	1.25	0.625
1b	21.5	22.5	1.25	1.25	2.50	1.25
1c	23.5	20.0	2.50	2.50	5.00	2.50
CPFX	37.0	32.0	0.156	0.156	1.25	0.625

Key: CPFX = Ciprofloxacin

3.3. Conflict of Interests

The authors declare that they do not have any financial relations with any of the commercial entities mentioned in the paper that could lead to a conflict of interests.

4. ACKNOWLEDGMENT

We wish to thank the College of Agriculture, Engineering and Science of the University of KwaZulu-Natal, Durban, South Africa for running the NMR analysis.

REFERENCES

- [1] M. Raghav, T. Isha, and K. Sachin, "Synthesis and antimicrobial evaluation of novel thiophene derivatives," *Der Pharmacia Sinica*, vol. 3, pp. 332-336, 2012.
- [2] I. C. F. Ferreira, R. C. Calhelha, L. M. Estevinho, and M. J. R. Queiroz, "Screening of antimicrobial activity of diaryl amine in the 2,3,5-trimethyl benzo [b] thiophene series: A structure activity evaluation study," *Bioorg.Med.Chem.Lett.*, vol. 14, pp. 5831-5833, 2004.
- [3] U. V. Laddi, M. B. Talwar, S. R. Desai, Y. S. Somannavar, R. S. Bennur, and S. C. Bennur, "Antiinflammatory activity of 3-substituted-4-amino-5-piperidino-4 (H)-1, 2, 4-triazoles," *Indian Drugs*, vol. 35, pp. 509-513, 1998.
- [4] C. F. R. Ferreira, R. P. Maria-Joao, M. Vilas-Boas, L. M. Estevinho, A. Begouin, and K. Gilbert, "Evaluation of the antioxidant properties of diarylamines in the benzo[b]thiophene series by free radical scavenging activity and reducing power," *Bioorg. Med. Chem. Lett.*, vol. 16, p. 1384-1387, 2006.

- [5] I. Jarak, I. M. Kralj, M. L. Suman, L. G. Pavlovic, G. J. Dogan, and J. I. Piantanida, "Novel cyano and nisopropylamidino-substituted derivatives of benzo[b]thiophen-2-carboxanilides and benzo[b]thieno[2,3-c] quinolones: Synthetic, photochemical synthesis, crystal structure determination and antitumor evaluation," *J. Med. Chem.*, vol. 48, pp. 2346-2360, 2005.
- [6] A. K. Gadad, H. Kumar, C. J. Shishoo, I. Mkhazi, and C. S. Mahajanshetti, "Synthesis of some 2-aminoacetyl-amino-3-carboethoxy/anilido-4, 5,6,7-tetrahydrobenzo [b] thiophenes for local anesthetic activity," *Ind. J. Chem. Soc.*, vol. 33, pp. 298-301, 1994.
- [7] E. Gillespie, K. M. Dungan, A. W. Gomol, and R. J. Seidehamel, "Selected immunologic properties of tiprinast, a non-steroidal antiallergy agent," *Int. J. Immunopharmac.*, vol. 7, p. 655-660, 1985.
- [8] O. O. Aboaba, S. I. Smith, and F. O. Olude, "Antibacterial effect of edible plant extract on escherichia coli 0157: H7," *Pak. J. Nutr.*, vol. 5, pp. 325 – 327, 2006.
- [9] H. Seifert, C. Von Eiff, and G. Fatkenneuer, "Fatal case due to methicillin-resistant staphylococcus aureus small colony variants in an AIDS patient," *Emerg. Infect. Dis.*, vol. 5, pp. 450 – 453, 1999.
- [10] C. Von Eiff, D. Bettin, R. A. Proctor, B. Rolauuffs, N. Lindner, W. Winkelmann, and G. Peters, "Recovery of small colony variants of Staphylococcus aureus following gentamicin bead placement for osteomyelitis," *Clin. Infect. Dis.*, vol. 25, pp. 1250 – 1251, 1997.
- [11] B. Kahl, M. Herrmann, A. S. Everding, H. G. Koch, K. Becker, E. Harms, R. A. Proctor, and G. Peters, "Persistent infection with small colony variant strains of Staphylococcus aureus in patients with cystic fibrosis," *J. Infect. Dis.*, vol. 177, p. 1023-1029, 1998.
- [12] M. Abele-Horn, B. Schupfner, P. Emmerling, H. Waldner, and H. Goring, "Persistent wound infection after herniotomy associated with small-colony variants of Staphylococcus aureus," *Infection*, vol. 28, p. 53-54, 2000.
- [13] T. Ojala, S. Remes, P. Haansuu, H. Vuorela, R. Hiltunen, K. Haahtela, and P. Vuorela, "Antimicrobial activity of some coumarin containing herbal plants growing in Finland," *J. Ethnopharmacol.*, vol. 73, pp. 299 – 305, 2000.
- [14] J. Klein, R. Feigin, and G. Mc Cracken, "Report of the task force on diagnosis and management of meningitis," *Pediatrics*, vol. 78, pp. 959-982, 1986.
- [15] M. Falagas and S. Gorbach, "Practice guidelines: Urinary tract infections," *Infect. Dis. Clin. Pract.*, vol. 4, p. 241-245, 1955.
- [16] K. Gewald, E. Schinke, and H. Bottcher, "Heterocycles from C-H acidic nitriles, VIII - 2 – amino – thiophene of methylene – active nitriles, carbonyl compounds and sulphur," *Chem. Ber.*, vol. 99, p. 94-100, 1966.
- [17] A. S. Abd-El-Aziz and T. H. Afifi, "Organoiron polynorbornenes with pendent arylazo and hetarylazo dye moieties," *Macromolecular Symposia*, vol. 209, pp. 195-206, 2006.
- [18] D. A. Karou, A. Savadoga, A. Canini, S. Yameoga, and C. Montesano, "Anti-bacterial activity of alkaloids from Sida acuta," *Afri. J. Biotechnol.*, vol. 5, pp. 195 – 200, 2006.

Views and opinions expressed in this article are the views and opinions of the author(s), International Journal of Chemistry and Materials Research shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/arising out of the use of the content.