DESIGN AND ANTIMICROBIAL ACTIVITY EVALUATION OF NEW PYRAZOLE, AND 1, 3, 4-THIADIAZOL DERIVATIVES WITH NICOTINOYL MOIETY

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Abstract

Novel 1, 3, 4-Thiadiazol derivatives, New Pyrazole, and related substances in synthesised form, bearing nicotinoyl moiety. By using elemental analysis, FT-IR, 1H NMR, and 13C NMR, the structures of all the synthesised compounds have been verified. With ampicillin as a reference antibiotic, freshly synthesised compounds were tested in vitro for antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus pyogenes*. Good bacterial activity was reported for compounds 6e and 7a. Activities against the tested strains of bacteria and fungus were reported to be inconsistent and low.

Keywords: Thiadiazol derivatives, analysis, FT-IR, 1H NMR, and 13C NMR

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Introduction

Despite ongoing research devoted to the discovery and development of new anti- microbial drugs, the frequency of microbial illnesses is rising globally. Various vaccinations against bacterial and acute viral infections were created and made widely accessible throughout the last ten years. [1, 2]. A significant medical issue is the spread of pathogenic bacterial and fungal strains that are resistant to the antimicrobial medicines that are currently on the market. Therefore, it is imperative for medicinal chemists to create novel antimicrobial drugs with lower toxicity and greater effects in a shorter amount of time. [3]. The majority of medications on the market today are based on heterocyclic compounds, or substances having rings that contain one or more atoms other than carbon. [4].

Heterocyclic compounds, particularly the N-heterocycles, are the most influential class of compounds in the pharmaceutical industry with a strong preponderance (~ 60%) among the drug candidate molecules [5]. Among many classes of heterocyclic units, nicotinyl ring system has been widely used as a component of different antibiotic molecules. Remarkable examples include hinomycin and levomycin that are inhibitors of gram-positive bacteria and active against various transplantable tumors [6]. The large spectrum of biological activities of nictonyl based compounds and their great practical usefulness continues to encourage medicinal chemist to design new and more efficient synthetic methodologies. Several new approaches were realized to the current existing drugs to reduce the microbial resistance. These, for the most part, require structural modification of actual antimicrobial agents to enhance the microbial intracellular concentration of drug, and thereby to boost antimicrobial activity. The throughout literature surveys indicates that the structural scaffold that includes hydrazide nitrogens double-bonded to carbon atoms (-C=N–NH-), displays potent antimicrobial activity [7, 8]. The spectrum of activities that Schiff base hydrazides exhibit, which involves anticancer, antimicrobial effects, etc. make them relevant organic scaffolds [9,10]. During the past years, substituted 1,3,4-thiadiazole derivatives have gained attention and have been progressively explored as a result of their broad spectrum of pharmacological properties. It is assumed that 1,3,4-thiadiazole derivatives exhibit numerous biological activities due to the presence of =N–C–S– moiety [11]. Other studies showed that the biological activities of 1,3,4-thiadiazole derivatives are the result of the important aromaticity of the ring, which also bring significant in- vivo stability to this five-membered ring. Keeping in view of the biological importance of Nicotine, hydrazide and 1,3,4-thiadiazoles derivatives and in continuation to our research plan aiming to design potent bioactive agents [12-14], we report herein the synthesis, characterization and in vitro biological activity of new Pyrazoles, 1,2,4-triazole and 1,3,4-thiadiazol derivatives possessing 1,4-dihydronicotinyl moiety. The chemical structures of the novel heterocyclic compounds were determined based on IR, 1H and 13C NMR and MS spectral data. The synthesized compounds were screened in vitro for their antibacterial activities against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa bacteria.

Experimental section

Melting points were calculated using a Weiss-Gallenkamp Electro Thermal 9100 melting point equipment (Loughborough, UK). Using Kieselgel 60 F254 (Merck), Thin-Layer Chromatography (TLC) was used to verify the compounds' purity. On a JEOL JMS 600 mass spectrometer, EI-MS was carried out. Bruker 1H-NMR signals showed at 300 and 400 MHz in CH3OH-d4 and at 75 MHz in CHCl3-d4 respectively. On a Shimadzu, IR Prestige- 21 (Shimadzu, Tokyo, Japan), Fourier-transform infrared spectroscopy (FT-IR) spectra were acquired.

General procedure for the synthesis of 6-Aryl-2-methylnicotinohydrazides.3(a-f)

A mixture of the appropriate ester (5 mmol) and 99% hydrazine hydrate (5 mL) was refluxed for 3 h. The solid product obtained upon cooling was filtered off and recrystallized from dioxane to afford the corresponding 6-aryl-2-methylnicotino hydrazides respectively.

General procedure for the synthesis of (E)-3-(((2-substituted phenyl hydrazineyl)oxy) carbonyl) -2-methyl-6-phenylpyridine.4(a-l)

A mixture of substituted benzaldehyde (0.35 g, 2.5 mmol) and hydrazides (0.34 g, 2.5 mmol) in 50 mL of methanol was refluxed with continuous stirring for 3 h. The obtained solution was cooled and after several days colourless crystalline product

General procedure for the synthesis of Ethyl(E)-4-(2-((2-methyl-6-susbstituted phenyl nicotinoyl)oxy)hydrazineylidene)pentanoate .5(a-f)

A mixture of compound 3(a-f) (0.01 mol) and ethyl acetoacetate (0.01 mol) in acetic acid (10 mL) was refluxed for 5 h, cooled and the reaction mixture was poured onto ice-water to give a pale

yellow powder, which was subsequently crystallized from ethanol.

General procedure for the synthesis of 3-(((6-chloro-2-methylnicotinoyl)oxy)amino)-2-(5-(4-chlorophenyl)furan-2-yl)thiazolidin-4-one.6(a-l)

A mixture of compounds (IV–VI) (0.01 mol) and thioglycolic acid (0.01 mol) in dry pyridine (10 mL) was refluxed for 6 h, cooled, and the reaction mixture was poured onto cold diluted HCl solution. The obtained solid was filtered off, and crystallized from ethanol.

General procedure for the synthesis of 5-methyl -2-(2-methyl-6-phenylsubstituted nicotinoyl)-2,4-dihydro-3H-pyrazol-3-one 7(a-f)

A solution of compound 5(a-f) (3.17 g, 0.01 mol) in sodium hydroxide (10%, 20 mL) was boiled under reflux for 6 h, then the reaction mixture was poured onto ice-water and neutralized with diluted Hcl. The obtained solid was collected by filtration, washed with water and crystallized from methanol to afford green crystals.

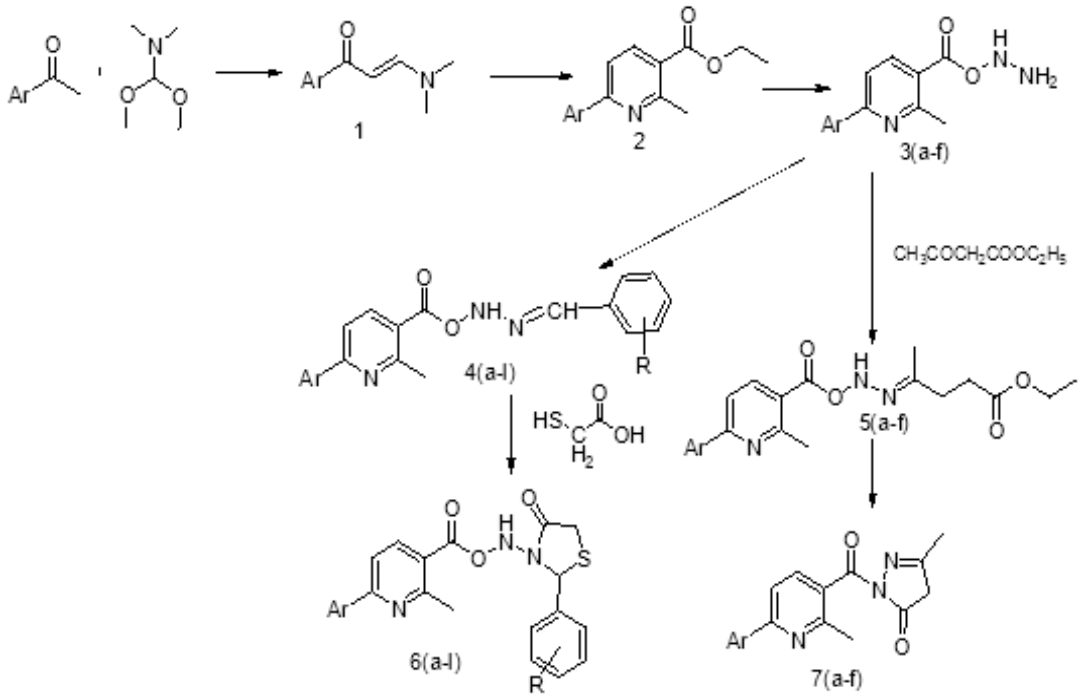

Figure 1: Scheme for synthesis of compound

Spectral data of synthesised compounds

Compound 1a

^{1}H NMR: δ 2.89 (6H, s), 5.53 (1H, d, *J* = 16.7 Hz), 7.46-7.61 (3H, 7.53 (d, *J* = 7.6, Hz), 7.54 (d, *J* = 8.5, 7.2, Hz)), 7.75 (2H, d, *J* = 8.5, 0.4 Hz), 8.18 (1H, d, *J* = 16.7 Hz).

^{13}C NMR: δ 41.3 (2C, s), 89.3 (1C, s), 127.8 (1C, s), 128.4 (2C, s), 129.0 (2C, s), 135.5 (1C, s), 154.8 (1C, s), 183.6 (1C, s).

Compound 2a

^{1}H NMR: δ 1.30 (3H, t, *J* = 7.1 Hz), 2.69 (3H, s), 4.10 (2H, q, *J* = 7.1 Hz), 7.36-7.61 (3H, 7.42 (t, *J* = 7.5Hz), 7.54 (d, *J* = 7.9, Hz)), 7.86 (2H, d, *J* = 7.9Hz), 8.03 (1H, d, *J* = 6.1 Hz), 8.23 (1H, d, *J* = 6.1 Hz).

^{13}C NMR: δ 41.3 (2C, s), 89.3 (1C, s), 127.8 (1C, s), 128.4 (2C, s), 129.0 (2C, s), 135.5 (1C, s), 154.8 (1C, s), 183.6 (1C, s).

Compound 3a

^{1}H NMR: δ 2.75 (3H, s), 7.36-7.61 (3H, 7.42 (t, *J* = 7.5, 1.4 Hz), 7.54 (d, *J* = 7.8, Hz)), 7.78 (2H, d, *J* = 7.8, 1.5Hz), 8.04 (1H, d, *J* = 8.0 Hz), 8.24 (1H, d, *J* = 8.0 Hz)

Compound 4a

^{1}H NMR: δ 2.69 (3H, s), 7.20-7.62 (8H, 7.26 (t, *J* = 7.4, Hz), 7.36 (d, *J* = 7.9Hz), 7.40 (d, *J* = 7.9Hz), 7.42 (t, *J* = 7.5Hz), 7.54 (d, *J* = 7.8Hz)), 7.78 (2H, d, *J* = 7.8Hz), 7.89 (1H, s), 8.04 (1H, d, *J* = 8.0 Hz), 8.24 (1H, d, *J* = 8.0 Hz).

Compound 5a

^{1}H NMR: δ 1.15 (3H, t, *J* = 7.1 Hz), 1.75 (2H, quint, *J* = 7.4 Hz), 2.01 (3H, s), 2.21-2.34 (4H, 2.27 (t, *J* = 7.4 Hz), 2.28 (t, *J* = 7.4 Hz)), 2.69 (3H, s), 4.12 (2H, q, *J* = 7.1 Hz), 7.36-7.62 (3H, 7.42 (t, *J* = 7.5, Hz), 7.54 (d, *J* = 7.8Hz)), 7.78 (2H,

d, *J* = Hz), 7.98-8.17 (2H, 8.04 (d, *J* = 8.4 Hz), 8.11 (d, *J* = 8.4 Hz)).

Compound 6a
^{1}H NMR: δ 2.69 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.75 (1H, d, *J* = 16.0 Hz), 6.17 (1H, s), 7.27-7.62 (8H, 7.33 (d, *J* = 7.9Hz), 7.34 (t, *J* = 7.7, Hz), 7.42 (t, *J* = 7.5Hz), 7.45 (d, *J* = 7.9Hz), 7.54 (d, *J* = 7.8, Hz)), 7.78 (2H, d, *J* = 7.8, Hz), 8.04 (1H, d, *J* = 7.9 Hz), 8.24 (1H, d, *J* = 7.9 Hz).

Compound 6b
^{1}H NMR: δ 2.28 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.74 (1H, d, *J* = 16.0 Hz), 6.15 (1H, s), 7.08 (1H, d, *J* = 8.6Hz), 7.27-7.78 (10H, 7.33 (d, *J* = 7.9, Hz), 7.34 (t, *J* = 7.7, Hz), 7.45 (d, *J* = 7.9Hz), 7.44 (d, *J* = 8.6Hz), 7.53 (d, *J* = 8.7Hz), 7.52 (d, *J* = 4.2Hz), 7.64 (d *J* = 8.7, 0.4 Hz), 7.72 (d, *J* = 2.0, 0.4 Hz)).
^{13}C NMR: δ 20.0 (1C, s), 33.6 (1C, s), 65.2 (1C, s), 123.9 (1C, s), 124.5 (1C, s), 127.4-127.5 (2C, 127.4 (s), 127.5 (s)), 127.7-127.8 (3C, 127.8 (s), 127.8 (s), 127.8 (s)), 128.2 (2C, s), 128.4 (2C, s), 128.9 (1C, s), 135.1 (1C, s), 136.0 (1C, s), 139.3 (1C, s), 143.2 (1C, s), 161.0 (1C, s), 168.7 (1C, s).

Compound 6c
^{1}H NMR: δ 2.39 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.74 (1H, d, *J* = 16.0 Hz), 6.15 (1H, s), 7.27-7.59 (11H, 7.33 (d, *J* = 7.9, Hz), 7.34 (t, *J* = 7.7Hz), 7.36 (d, *J* = 8.7, Hz), 7.45 (d, *J* = 7.9, Hz), 7.45 (d, *J* = 8.7, Hz), 7.46 (d, *J* = 8.8 Hz), 7.53 (d, *J* = 8.8 Hz)), 7.67-7.89 (3H, 7.72 (d, *J* = 1.9, 0.4 Hz), 7.74 (d, *J* = 7.1, 0.4 Hz), 7.83 (d, *J* = 7.1, 1.9 Hz)).
^{13}C NMR: δ 20.0 (1C, s), 33.6 (1C, s), 65.2 (1C, s), 123.9-124.0 (3C, 123.9 (s), 124.0 (s), 124.0 (s)), 127.4 (1C, s), 127.6 (2C, s), 127.8-127.8 (2C, 127.8 (s), 127.8 (s)), 128.2 (2C, s), 128.4 (2C, s), 128.7 (2C, s), 128.9 (1C, s), 133.7 (1C, s), 134.3 (1C, s), 135.1 (1C, s), 136.0 (1C, s), 139.3 (1C, s), 151.0-151.2 (2C, 151.1 (s), 151.1 (s)), 161.0 (1C, s), 168.7 (1C, s).

Compound 6d
^{1}H NMR: δ 2.40 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.74 (1H, d, *J* = 16.0 Hz), 6.15 (1H, s), 7.20 (2H, d, *J* = 8.7, Hz), 7.27-7.59 (9H, 7.33 (d, *J* = 7.9, Hz), 7.34 (t, *J* = 7.7Hz), 7.36 (d, *J* = 8.7, 1.5, 0.5 Hz), 7.42 (d, *J* = 8.8 Hz), 7.45 (d, *J* = 7.9Hz), 7.53 (d, *J* = 8.8 Hz)), 7.65-7.80 (2H, 7.71 (d, *J* = 1.9, Hz), 7.74 (d, *J* = 7.1Hz)), 7.90 (1H, d, *J* = 7.1, Hz).
^{13}C NMR: δ 20.0 (1C, s), 33.6 (1C, s), 65.2 (1C, s), 122.3 (1C, s), 123.9-124.0 (3C, 123.9 (s), 124.0 (s), 124.0 (s)), 127.4 (1C, s), 127.8-127.8 (2C, 127.8 (s), 127.8 (s)), 127.9 (2C, s), 128.2 (2C, s), 128.4 (2C, s), 128.9 (1C, s), 131.7 (2C, s), 134.3 (1C, s), 135.1 (1C, s), 136.0 (1C, s), 139.3 (1C, s), 151.0-151.2 (2C, 151.1 (s), 151.1 (s)), 161.0 (1C, s), 168.7 (1C, s).

Compound 6e
^{1}H NMR: δ 2.31 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.74 (1H, d, *J* = 16.0 Hz), 6.15 (1H, s), 6.67 (2H, d, *J* = 8.9, 1.4Hz), 7.19-7.54 (9H, 7.25 (d, *J* = 8.6 Hz), 7.33 (d, *J* = 7.9, 1.5, Hz), 7.34 (t, *J* = 7.7Hz), 7.39 (d, *J* = 8.9, Hz), 7.45 (d, *J* = 7.9, 7.7Hz), 7.48 (d, *J* = 8.6 Hz)), 7.55-7.79 (3H, 7.62 (d, *J* = 8.4, Hz), 7.66 (d, *J* = 8.4, Hz), 7.74 (d, *J* = 2.0, Hz)).
^{13}C NMR: δ 20.0 (1C, s), 33.6 (1C, s), 65.2 (1C, s), 114.3 (2C, s), 123.9-124.0 (3C, 123.9 (s), 124.0 (s), 124.0 (s)), 127.4 (1C, s), 127.8-127.8 (2C, 127.8 (s), 127.8 (s)), 128.2 (2C, s), 128.4 (2C, s), 128.6 (2C, s), 128.9 (1C, s), 134.3 (1C, s), 135.1 (1C, s), 136.0 (1C, s), 139.3 (1C, s), 148.4 (1C, s), 151.0-151.2 (2C, 151.1 (s), 151.1 (s)), 161.0 (1C, s), 168.7 (1C, s).

Compound 6f
^{1}H NMR: δ 2.40 (3H, s), 3.60 (1H, d, *J* = 16.0 Hz), 3.74 (1H, d, *J* = 16.0 Hz), 6.15 (1H, s), 7.20 (2H, d, *J* = 8.7Hz), 7.27-7.59 (9H, 7.33 (d, *J* = 7.9, Hz), 7.34 (t, *J* = 7.7, Hz), 7.36 (d, *J* = 8.7, Hz), 7.42 (d, *J* = 8.8 Hz), 7.45 (d, *J* = 7.9Hz), 7.53 (d, *J* = 8.8 Hz)), 7.65-7.80 (2H, 7.71 (d, *J* = 1.9, 0.4 Hz), 7.74 (d, *J* = 7.1, 0.4 Hz)), 7.90 (1H, d, *J* = 7.1, 1.9 Hz).
^{13}C NMR: δ 20.0 (1C, s), 33.6 (1C, s), 65.2 (1C, s), 122.3 (1C, s), 123.9-124.0 (3C, 123.9 (s), 124.0 (s), 124.0 (s)), 127.4 (1C, s), 127.8-127.8 (2C, 127.8 (s), 127.8 (s)), 127.9 (2C, s), 128.2 (2C, s), 128.4 (2C, s), 128.9 (1C, s), 131.7 (2C, s), 134.3 (1C, s), 135.1 (1C, s), 136.0 (1C, s), 139.3 (1C, s), 151.0-151.2 (2C, 151.1 (s), 151.1 (s)), 161.0 (1C, s), 168.7 (1C, s).

Compound 7a
^{1}H NMR: δ 1.49 (3H, d, *J* = 7.5 Hz), 2.06-2.16 (3H, 2.11 (s), 2.11 (s), 2.11 (s)), 2.64-2.74 (3H, 2.69 (s), 2.69 (s), 2.69 (s)), 3.29 (2H, d, *J* = 16.3 Hz), 4.70-4.85 (2H, 4.77 (d, *J* = 16.9, 2.6 Hz), 4.77 (d, *J* = 10.1, 2.6 Hz)), 5.59 (1H, d, *J* = 16.9, 10.1, 7.5 Hz), 7.35-7.61 (3H, 7.42 (tt, *J* = 7.5, 1.4 Hz), 7.54 (d, *J* = 7.8, 7.5, 1.4, 0.5 Hz), 7.54 (d, *J* = 7.8, 7.5, 1.4, 0.5 Hz)), 7.78 (1H, d, *J* = 7.8, 1.5, 0.5 Hz), 7.86-8.01 (2H, 7.92 (d, *J* = 7.9 Hz), 7.94 (d, *J* = 7.8, 1.5, 0.5 Hz)), 8.16 (1H, d, *J* = 7.9 Hz).

Compound 7b
^{1}H NMR: δ 2.11 (3H, s), 2.63 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 6.69 (1H, d, *J* = 3.4, Hz), 7.28 (1H, d, *J* = 3.4, Hz), 8.07 (1H, d, *J* = 1.8, Hz), 8.20 (1H, d, *J* = 8.5 Hz), 9.02 (1H, d, *J* = 8.5 Hz).
^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 112.0 (1C, s), 112.6 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 127.8 (1C, s), 143.6 (1C, s),

152.4 (1C, s), 154.3 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7c

^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 107.9 (1C, s), 112.3 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 127.4 (2C, s), 127.8 (1C, s), 128.7 (2C, s), 129.1 (1C, s), 133.7 (1C, s), 152.4 (1C, s), 154.3 (1C, s), 155.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).
^{1}H NMR: δ 2.11 (3H, s), 2.60 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.21 (1H, d, *J* = 4.5 Hz), 7.43-7.73 (5H, 7.49 (d, *J* = 4.5 Hz), 7.60 (d, *J* = 8.7, 1.5, 0.4 Hz), 7.67 (d, *J* = 8.7, 1.6, 0.4 Hz)), 8.18 (1H, d, *J* = 6.5 Hz), 8.97 (1H, d, *J* = 6.5 Hz).

Compound 7d

^{1}H NMR: δ 2.11 (3H, s), 2.48 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 6.98 (1H, d, *J* = 4.4 Hz), 7.39-7.55 (3H, 7.45 (d, *J* = 4.4 Hz), 7.49 (d, *J* = 8.9, Hz)), 7.67 (2H, d, *J* = 8.9, Hz), 8.13 (1H, d, *J* = 6.6 Hz), 9.01 (1H, d, *J* = 6.6 Hz).
^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 107.9 (1C, s), 112.3 (1C, s), 117.7 (2C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 126.0 (2C, s), 127.8 (1C, s), 129.1 (1C, s), 139.5 (1C, s), 152.4 (1C, s), 154.3 (1C, s), 155.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7e

^{1}H NMR: δ 2.11 (3H, s), 2.61 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.05 (1H, d, *J* = 4.5 Hz), 7.49 (1H, d, *J* = 4.5 Hz), 7.59-7.74 (4H, 7.66 (d, *J* = 8.5, Hz), 7.67 (d, *J* = 8.5, Hz)), 8.16 (1H, d, *J* = 5.0 Hz), 8.97 (1H, d, *J* = 5.0 Hz)

^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 107.9 (1C, s), 112.3 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 122.3 (1C, s), 126.6 (2C, s), 127.8 (1C, s), 129.1 (1C, s), 131.7 (2C, s), 152.4 (1C, s), 154.3 (1C, s), 155.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7f

^{1}H NMR: δ 2.11 (3H, s), 2.60 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.20-7.36 (2H, 7.25 (d, *J* = 4.4 Hz), 7.30 (d, *J* = 8.1, Hz)), 7.39-7.54 (2H, 7.45 (td, *J* = 8.1Hz), 7.49 (d, *J* = 4.4 Hz)), 7.62 (1H, d, *J* = 8.1, Hz), 7.99 (1H, td, *J* = 1.6, Hz), 8.20 (1H, d, *J* = 6.5 Hz), 8.98 (1H, d, *J* = 6.5 Hz).

^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 107.9 (1C, s), 112.3 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 125.2 (1C, s), 125.7 (1C, s), 127.0 (1C, s), 127.8 (1C, s), 128.7 (1C, s), 130.4 (1C, s), 130.5 (1C, s), 152.4 (1C, s), 154.3 (1C, s), 155.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s)

Compound 7g

^{1}H NMR: δ 2.11 (3H, s), 2.60 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.17 (1H, d, *J* = 8.3, 4.2 Hz), 7.61 (1H, d, *J* = 8.3, Hz), 7.80 (1H, d, *J* = 4.2Hz), 8.22 (1H, d, *J* = 9.3 Hz), 8.40 (1H, d, *J* = 9.3 Hz).
^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 127.2 (1C, s), 127.5 (1C, s), 127.7-127.8 (2C, 127.8 (s), 127.8 (s)), 144.8 (1C, s), 152.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7h

^{1}H NMR: δ 2.11 (3H, s), 2.60 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.38 (2H, d, *J* = 8.7, Hz), 7.45-7.61 (3H, 7.51 (d, *J* = 8.7, Hz), 7.55 (d, *J* = 8.7 Hz)), 7.73 (1H, d, *J* = 8.7 Hz), 8.20 (1H, d, *J* = 5.5 Hz), 8.45 (1H, d, *J* = 5.5 Hz).
^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 124.0 (1C, s), 124.4 (1C, s), 127.6 (2C, s), 127.8 (1C, s), 128.7 (2C, s), 133.7 (1C, s), 134.3 (1C, s), 148.8 (1C, s), 151.1 (1C, s), 152.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7i

^{1}H NMR: δ 2.11 (3H, s), 2.51 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 6.69 (2H, d, *J* = 8.9, Hz), 7.37-7.52 (3H, 7.44 (d, *J* = 8.9, Hz), 7.46 (d, *J* = 8.6 Hz)), 7.63 (1H, d, *J* = 8.6 Hz), 8.09 (1H, d, *J* = 5.8 Hz), 8.97 (1H, d, *J* = 5.8 Hz).
^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 114.3 (2C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 124.0 (1C, s), 124.4 (1C, s), 127.8 (1C, s), 128.6 (2C, s), 134.3 (1C, s), 148.4 (1C, s), 148.8 (1C, s), 151.1 (1C, s), 152.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Compound 7j

^{1}H NMR: δ 2.11 (3H, s), 2.61 (3H, s), 3.29 (2H, d, *J* = 16.3 Hz), 7.30 (1H, d, *J* = 8.1, Hz), 7.42-7.63 (2H, 7.48 (t, *J* = 8.1, Hz), 7.56 (d, *J* = 8.1, Hz)), 7.63-7.80 (2H, 7.70 (d, *J* = 8.7 Hz), 7.74 (d, *J* = 8.7 Hz)), 8.04 (1H, t, *J* = 1.6, Hz), 8.22 (1H, d, *J* = 5.5 Hz), 8.46 (1H, d, *J* = 5.5 Hz).

^{13}C NMR: δ 16.0 (1C, s), 24.3 (1C, s), 43.1 (1C, s), 121.9-122.0 (2C, 121.9 (s), 121.9 (s)), 124.0 (1C, s), 124.4 (1C, s), 127.0 (1C, s), 127.7-127.8 (2C, 127.7 (s), 127.8 (s)), 128.7 (1C, s), 128.9 (1C, s), 130.4 (1C, s), 133.0 (1C, s), 148.8 (1C, s), 151.1 (1C, s), 152.2 (1C, s), 156.3 (1C, s), 163.0 (1C, s), 164.9 (1C, s), 170.6 (1C, s).

Spectras of Synthesised Compounds

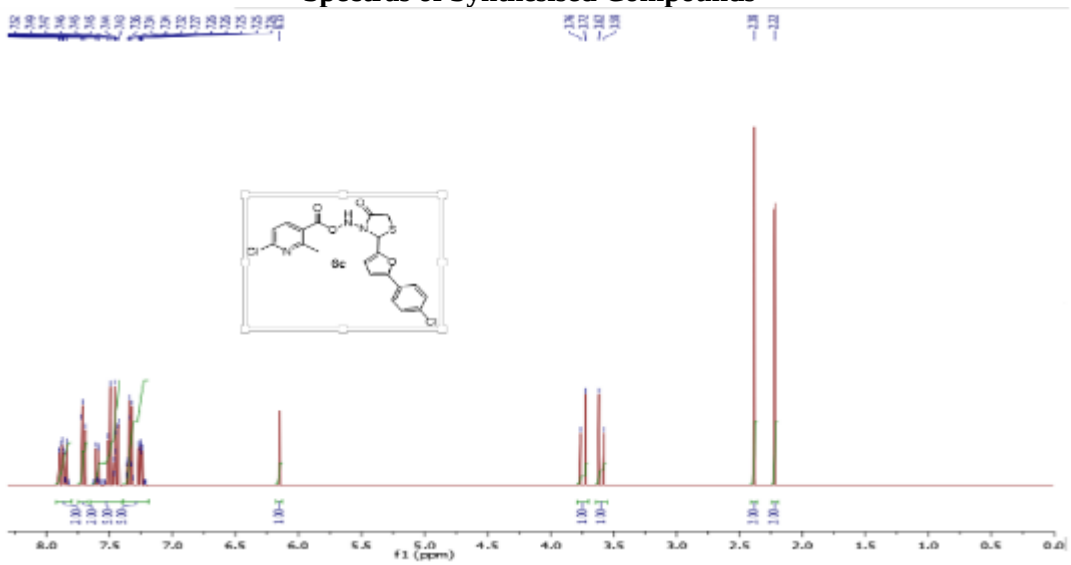

Figure 2: 1HNMR Spectra of Compound 6c

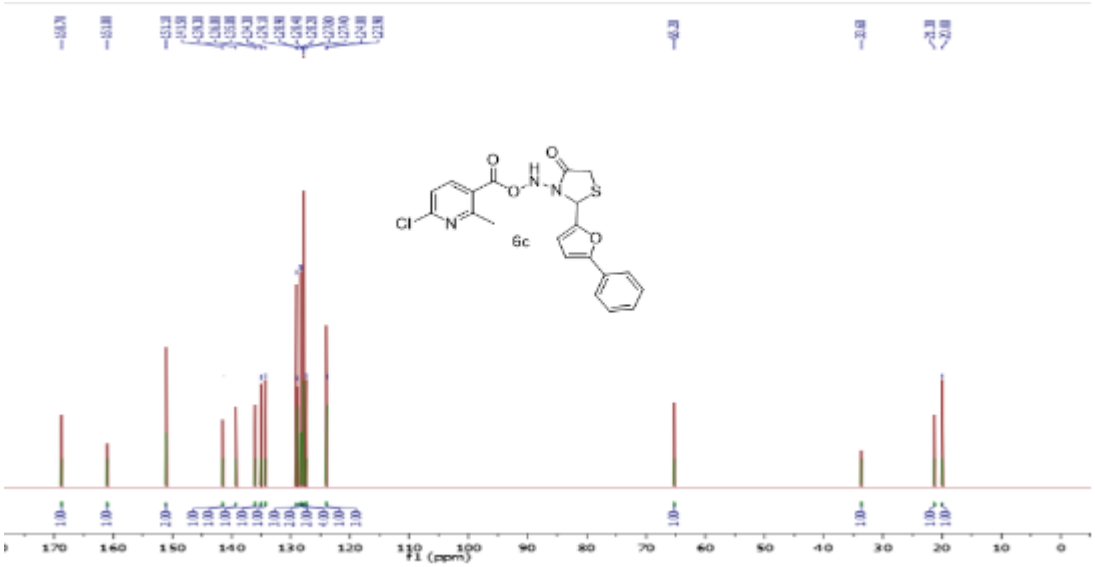

Figure 3: C13 NMR Spectra of Compound 6c

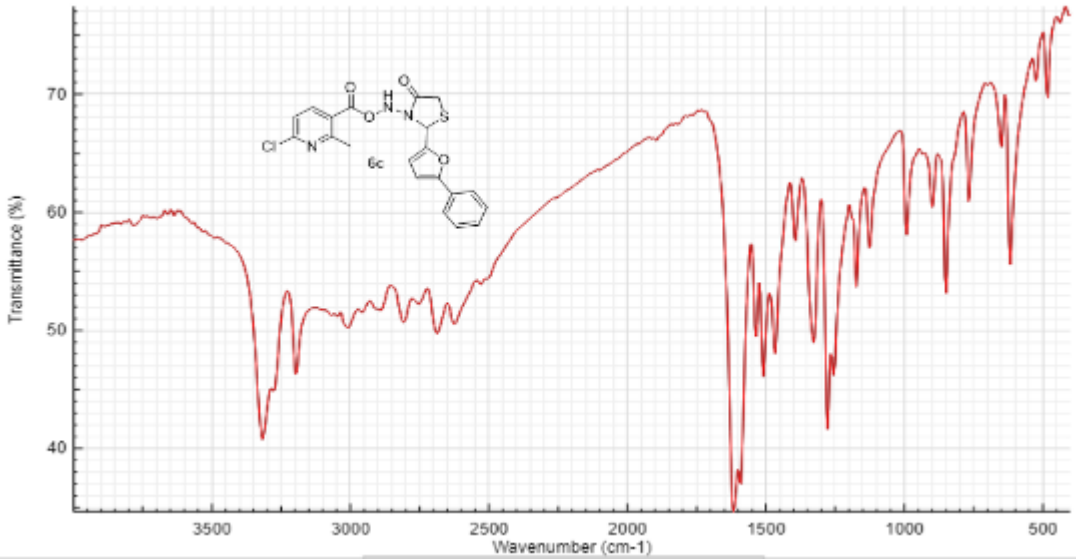

Fig 4: IR Spectra of Compound 6c

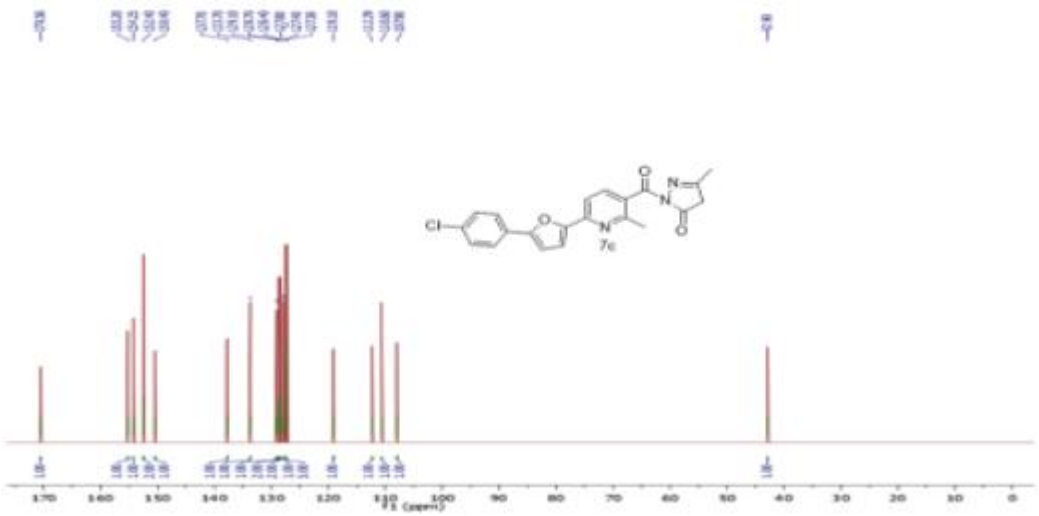

Fig 5 : 1HNMR Spectra of Compound 7c

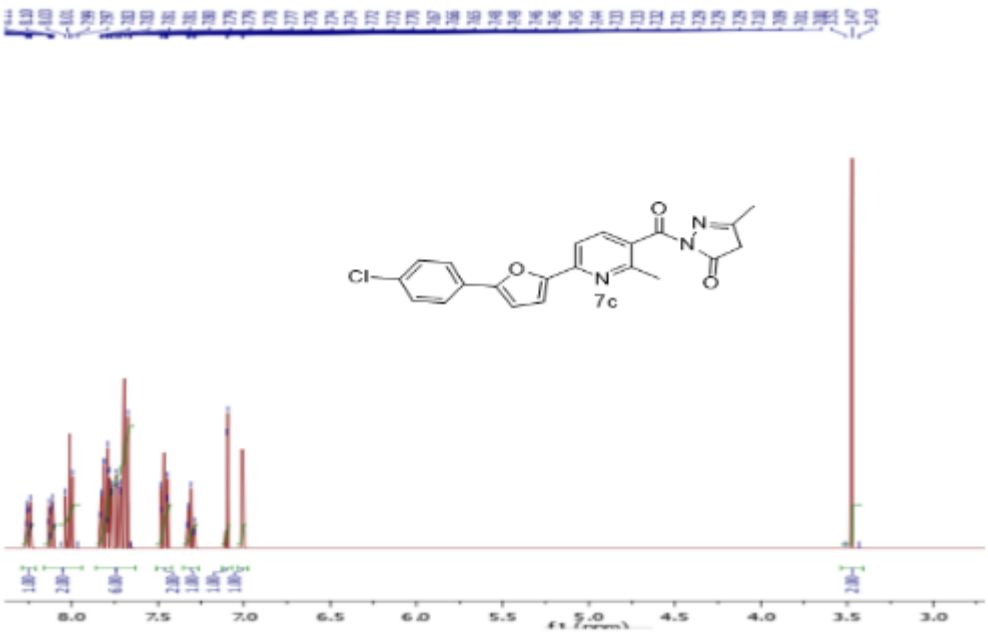

Fig 6: C13 NMR Spectra of Compound 7c

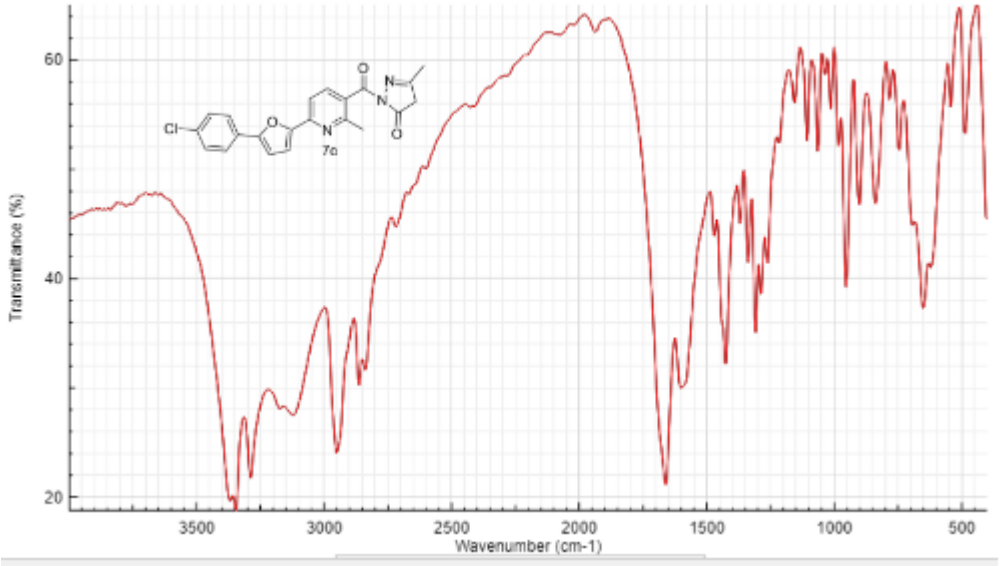

Fig 7: IR Spectra of Compound 7c

Pharmacological activity

Antibacterial activity

In vitro antibacterial activities of the test compounds against multi drug resistant bacterial strains were studied by disk diffusion assay [15]. The media was prepared by dissolving 38 g of Mueller Hinton agar (MHA) medium in 1000 mL of distilled water and autoclaved at 121 ℃ for 15 min. The autoclaved medium was poured into sterile plates (20–25 mL/plate) and allowed to solidify under sterile condition at room temperature. The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37 ℃ [16, 17]. Inoculated medium containing a 24 h grown culture was added aseptically to the nutrient medium and mixed systematically to get an even distribution. The solution was poured into ~20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature [16, 17]. Sterile agar-disc diffusion previously soaked in a known concentration (100 μg/mL and 200 μg/mL per disc) of the synthesized compound and standard drugs were prepared in DMSO using nutrient agar tubes and carefully placed at the center of the labeled seeded plate [16, 17]. Mueller–Hinton sterile agar plates were seeded with indicator bacterial strains (1.3×10^8 cfu/mL) and allowed to stay at 37 °C for 3 h. Sterile filter paper disks with a diameter of 6 mm were placed over these plates. Finally, the plates were incubated at 37 °C for 24 h [15,16,17].The mean inhibition zones were measured with a ruler and compared with the positive control (a disk containing amoxicillin) of the same concentration in millimeter. DMSO was used as a negative control during the whole test. The mean inhibition zone (MIZ) was expressed as mean value ± standard deviation.

Bacterial culture

The bacterial species, isolated from stored stool specimens and identified according to biochemical tests, were obtained from Adama Public Health Research & Referral Laboratory Center, Ethiopia. It is important to note that no patient was involved during specimen collection. The species were then cultured overnight using Eosin Methylene Blue (EMB) agar [17]. Gram-negative strains (*Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853)) and Gram-positive strains (*Staphylococcus aureus* (ATCC25923) and *Streptococcus pyogenes* (ATCC19615)) were used to test the activities of the synthesized compounds. Different concentrations were prepared from the synthesized compounds by dissolving 5 mg of each compound in 5 mL DMSO to make 1 mg/mL of standard solution. The experiment was performed in triplicates. The standard solution was serially diluted to furnish 100 μg/mL and 200 μg/mL samples for each synthesized compound. The concentrations of each sample were incorporated into sterile blank paper discs and dried at 37 ℃ [17].

Antibacterial activity of thiazoles

Thiazole containing compounds were proved to have potent antimicrobial activity against multidrug-resistant strains of *S. aureus* [18]. In this work, in vitro antibacterial activities of the synthesized compounds were done against four clinical bacterial isolates. The synthesized compounds were less active against Gram-negative rather than Gram-positive bacteria. Compounds **6e** and **6f** displayed good activities against *E. coli* with MIZ of 10.50 ± 0.02 and 14.40 ± 0.04 mm diameter, respectively, compared with amoxicillin (18.00 ± 0.01 mm) at 200 μg/mL. Compounds **6b** and **6f** showed good activities against *S. aureus*, while compounds **6a**, **6c** and **6d** showed good activities against *S. pyogenes* .

Compound **6e** showed strong antibacterial activity against Gram-negative (*P. aeruginosa*) and Gram-positive (*S. aureus*) with MIZ of 13.00 ± 0.02 and 15.00 ± 0.01 mm compared with amoxicillin (18.50 ± 0.45 and 17.00 ± 0.04 mm), respectively, at 200 μg/mL This suggests that compound **6e** is potentially a promising therapeutic antibacterial agent. As shown in table 1

Table 1: Zone of inhibition of the synthesised compound against microorganism

Compounds	**Conc.in μg/mL**		**Zone of inhibition (mm) mean ± SD**			
			E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenes***
6a	100		7.31 ± 0.03	8.00 ± 0.02	8.60 ± 0.02	7.95 ± 0.25
6b			7.45 ± 0.02	7.95 ± 0.30	7.20 ± 0.04	7.25 ± 0.01
6c			9.00 ± 0.01	10.00 ± 0.01	8.00 ± 0.04	8.67 ± 0.30
6d			8.00 ± 0.02	7.00 ± 0.01	7.00 ± 0.00	7.95 ± 0.25
6e			12.00 ± 0.01	12.43 ± 0.02	11.00 ± 0.00	13.00 ± 0.01
6f			8.02 ± 0.02	7.05 ± 0.01	7.05 ± 0.00	7.90 ± 0.25

Amoxicillin			12.00 ± 0.01	12.43 ± 0.02	11.00 ± 0.00	13.00 ± 0.01
6a	200		8.50 ± 0.01	9.80 ± 0.25	9.23 ± 0.02	10.03 ± 0.04
6b			9.25 ± 0.04	9.00 ± 0.01	9.80 ± 0.45	8.67 ± 0.25
6c			9.08 ± 0.01	9.43 ± 0.23	9.56 ± 0.25	9.89 ± 0.05
6d			10.50 ± 0.02	12.00 ± 0.01	11.25 ± 0.15	11.67 ± 0.30
6e			14.40 ± 0.04	13.00 ± 0.02	15.00 ± 0.01	14.45 ± 0.25
6f			14.38 ± 0.04	13.05 ± 0.02	15.10 ± 0.01	14.50 ± 0.25
Amoxicillin			18.00 ± 0.01	8.50 ± 0.45	17.00 ± 0.04	18.00 ± 0.02
Compounds	**Conc.in µg/mL**		**Zone of inhibition (mm) mean ± SD**			
			E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenes***
7a	100		8.02 ± 0.02	7.05 ± 0.01	7.05 ± 0.00	7.90 ± 0.25
7b			7.45 ± 0.02	7.95 ± 0.30	7.20 ± 0.04	7.25 ± 0.01
7c			9.00 ± 0.01	10.00 ± 0.01	8.00 ± 0.04	8.67 ± 0.30
7d			8.00 ± 0.02	7.00 ± 0.01	7.00 ± 0.00	7.95 ± 0.25
7e			12.00 ± 0.01	12.43 ± 0.02	11.00 ± 0.00	13.00 ± 0.01
7f			7.31 ± 0.03	8.00 ± 0.02	8.60 ± 0.02	7.95 ± 0.25
Amoxicillin			12.00 ± 0.01	12.43 ± 0.02	11.00 ± 0.00	13.00 ± 0.01
7a	200		14.40 ± 0.04	13.00 ± 0.02	15.00 ± 0.01	14.45 ± 0.25
7b			14.38 ± 0.04	13.05 ± 0.02	15.10 ± 0.01	14.50 ± 0.25
7c			9.08 ± 0.01	9.43 ± 0.23	9.56 ± 0.25	9.89 ± 0.05
7d			10.50 ± 0.02	12.00 ± 0.01	11.25 ± 0.15	11.67 ± 0.30
7e			8.50 ± 0.01	9.80 ± 0.25	9.23 ± 0.02	10.03 ± 0.04
7f			9.25 ± 0.04	9.00 ± 0.01	9.80 ± 0.45	8.67 ± 0.25
Amoxicillin			18.00 ± 0.01	8.50 ± 0.45	17.00 ± 0.04	18.00 ± 0.02

Compound **7a** showed strong antibacterial activity against Gram-negative (*P. aeruginosa*) and Gram-positive (*S. aureus*) with MIZ of 13.00 ± 0.02 and 15.00 ± 0.01 mm compared with amoxicillin (18.50 ± 0.45 and 17.00 ± 0.04 mm), respectively, at 200 µg/mL This suggests that compound **7a** is potentially a promising therapeutic antibacterial agent

Result and Discussion

Novel 1,3,4-Thiadiazol derivatives, New Pyrazole, and related substances In synthesised form, bearing nicotinoyl moiety. By using elemental analysis,. Compounds with chloro moiety showed good antibacterial activity. Structure of the compounds were confirmed by proton nmr, carbon -13 nmr and ir spectroscopy. And these molecules were sceened for antibacterial activity. With ampicillin as a reference antibiotic, freshly synthesised compounds were tested in vitro for antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus pyogenes. Good bacterial activity was reported for compounds 6e and 7a

Conclusions

The synthesized compounds showed moderate to high antibacterial activity. The in vitro antibacterial activity showed that compound **6e** is a promising antibacterial therapeutics agent against *E. coli.*

Conflict of Interests

The authors declare that they have no conflicts of interest

Author Contributions

All the authors contributed significantly to this manuscript, participated in reviewed and approved the final draft for publication.

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