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Original article

Synthesis, Characterization, and Antimicrobial Potential of Some Chlorinated Benzofuran Chalcones

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Abstract. *Study Objectives:* The reaction of 5-chlorosaliciylaldehyde with chloroacetone and potassium carbonate was used to prepare 1-(5-chloro-1-benzofuran-2-yl) ethanone (1) for starting reagent purposes. A new series of 1-(5-chloro-1-benzofuran-2-yl) ethanone-substituted chalcones 3a-i was synthesized using different substituted aromatic aldehydes in basic conditions by the Claisen–Schmidt condensation reaction. *Materials and Methods*: Structural analysis of the synthesized compounds was characterized by FT-IR and ^{1}H-^{13}C-NMR spectroscopy techniques. The antimicrobial properties of the chalcone derivatives (3a-i) were evaluated against the bacterial strains *Escherichia coli ATCC 25322, Klebsiella pneumoniae ATCC70060, Bacillus megaterium, Staphylococcus aureus* using the Agar well method. *Results:* New synthesized compounds demonstrated significant level antimicrobial activity against all bacteria. *Conclusion:* We described the synthesis of a new series of chloro-benzofuran chalcone hybrids as possible antibacterial agents in this paper.

Key words: Benzofuran, chalcone, antimicrobial effects, MBC, MIC, Chloro-benzofuran chalcone hybrids.

Introduction

Due to increased resistance to existing antibiotics for different reasons, scientists synthesize new types of compounds that work against multidrug-resistant pathogens. Molecular hybridization is a method commonly used to synthesize compounds that are biologically more active and effective while also performing some structural modifications. Chalcones are a class of natural products (1) with several biological and pharmacological activities (2-7). Chalcone molecules contain a carbonyl group and a double bond adjacent to this carbonyl group. Is known that chalcone molecules show biological activation because of the conjugation between the carbonyl group and this double bond (8-10). An additional area of interest is the combination of substitutes into the two aryl rings because this leads to a beneficial structure-activity relationship (SAR) and thus supports the synthetization of pharmacological active chalcones (11). Some chalcones exhibit antibacterial activities and thus have been identified as good candidates for use as antibiotics (12-17). The potential of these drugs has been confirmed by the fact that some chalcone-based drugs including metochalcone, sofalcone, and ilepcimide have been successfully developed and marketed.

In many biological evaluations, heterocyclic ring systems are known as powerful molecular structures (18), and heterocyclic compounds can support the creation of powerful and selective drugs (19). Benzofurans are one of the heterocyclic compound types with strong biological properties. Some benzofuran

ring systems with different substituents at C-2 positions are commonly found in nature, and bufuralol, amiodarone, and ailanthoidol are the most recognized and frequently mentioned benzofurans (Fig. 1). Ailanthoidol, obtained by isolating from the Chinese herbal medicine *Zanthoxylum ailanthoides*, is a neolignan bearing a 2-arylbenzofuran ring. Studies show that neolignans have properties including antioxidant, antiviral, anticancer, antifungal, and immunosuppressive activities (20). As reported in the literature, *amiodarone* is a highly effective antiarrhythmic compound used in the treatment of both supraventricular and ventricular arrhythmias (21). Bufuralol, a chiral molecule, contains asymmetric carbon. Bufuralol undergoes enantioselective and regioselective oxidation in the liver and is a good cytochrome P450 (CYP) substrate (22). We also previously reported that (benzofuran-2-yl) methanone derivatives showed strong inhibitory effects on cancer cells (23, 24).

The drugs with chlorine atoms have more lipophilicity since the drug-target interaction occurs more in the halogen-bound molecules (25, 26). In a very recent study, it was stated that chalcones carrying chlorine atoms have a stronger antibacterial activity against *S. aureus* than the standard drug; this indicates that chlorine attached to the phenyl ring increases antibacterial activity (27). According to another study, 2.4-diphenyl benzofuro [3,2-b] pyridines containing chlorine were highly effective in inhibiting topoisomerase activity (28). Considering these findings, a new series of 5-chloro-substituted benzofuran chalcones was designed.

We previously also reported some benzofuran linked chalcones as good antimicrobial compounds (29). Therefore, it was predicted that the conjugation of two bioactive entities like chalcone- and benzofuran-linked chloro may lead to better antimicrobial agents. Therefore, we planned to synthesize some chlorinated benzofuran-chalcone hybrids. In this work, we reported the synthesis of a new series of chloro-benzofuran chalcone hybrids as potential antimicrobial agents.

Materials and Methods

Melting points were measured using a differential scanning calorimeter (Shimadzu DSC-50) and are uncorrected. Elemental analyses were performed on a Leco CHNS-932 apparatus. NMR spectra were determined on a Bruker AC 400 (400-MHz) spectrometer with tetramethylsilane (TMS) as the internal standard with DMSO-d6 as the solvent. FT-Infrared (FT-IR) spectra were recorded as KBr pellets on a Perkin-Elmer Spectrum One FT-IR spectrometer.

Synthesis of the 1-(5-chloro-1-benzofuran-2-yl) ethanone (1)

5-chlorosaliciylaldehyde (3.91 g, 25 mmol), K2CO3 (4.84 g, 35 mmol), and dry acetone (100 mL) were placed in a 500 mL flask with a reflux condenser. The mixture was stirred at room temperature for 1 h. The Reaction mixture was cooled to 0-5oC, and then chloroacetone (1.99 mL 25 mmol) was added dropwise. The reaction mixture was stirred at room temperature for ten minutes and was refluxed. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was cooled, and then water was added. The solid was filtrated off and dried, and the compound (1) was crystallized from ethanol. The following product was obtained.

Yield: 72%, mp 100-101 oC IR spectrum, υ, cm-1: 1671 (C=O), 1547 (C=C). 1H NMR spectrum,

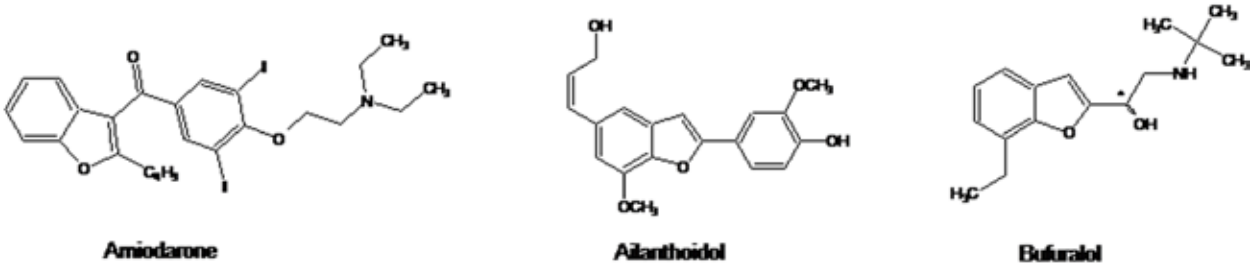

Figure 1. Benzofuran containing some biological molecules.

δ, ppm: 7.91 s (1H, 3-H), 7.83 s (1H, 4-H), 7.76 d (1H, 7-H, J=8.8 Hz), 7.55 d (1H, 6-H, J=8.0 Hz), 2.58 s (3H, COCH3). 13C NMR spectrum, δC, ppm: 188.39 (C=O), 153.87 (8-C), 153.75 (2-C), 128.88 (5-C), 128.81 (9-C), 128.78 (6-C), 123.34 (4-C), 114.45 (7-C), 113.91 (3-C), 26.96 (11-C). Calculated, %: C 61.70; H 3.60 Found, %: C 60.94; H 3.62.

General Procedure for synthesis of chalcones (3a-i)

A mixture of 1-(5-chloro-1-benzofuran-2-yl) ethanone (0.39 g, 2 mmol) and one of the aldehyde derivatives (2a-i, 2 mmol) was dissolved in 10 mL methanol. To this mixture, 3 mL aqueous sodium hydroxide (1 mol/L) was added at 0-5°C. The reaction mixture was stirred at room temperature for 3 h. Then, this reaction mixture was poured over crushed ice and acidified with HCl. The yellow solid obtained was filtered, washed with water, and dried. The residue was purified by crystallization from ethanol.

1-(5-chloro-1-benzofuran-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (3a)

Yield: 75%, mp 164-165 oC IR spectrum, υ, cm-1: 2832-2987 (C-H), 1653 (C=O), 1596 (C=C). 1H NMR spectrum, δ, ppm: 8.20 s (1H, Br3-H), 7.98 s (1H, Br4-H), 7.88 d (2H, Ar2,6-H, J=8.4 Hz), 7.82 d (1H, β-H, J=15.6 Hz), 7.80 d (1H, Br6-H, J=8,8 Hz), 7.74 d (1H, α-H, J=15.6 Hz), 7,58 d (1H, 7-H, J=8.8 Hz), 7.05 d (2H, Ar3,5-H, J=8.4 Hz), 3.84 s (3H, OCH3). 13C NMR spectrum, δC, ppm: 178.89 (C=O), 162.21 (Ar4-C) 155.05 (Br2-C), 154.14 (8-C), 144.47 (β-C), 131.52 (Br5-C), 129.07 (9-C), 128.86 (Br6-C), 128.83 (Ar1-C), 127.42 (Ar2,6-C), 123.35 (Br4-C), 119.64 (α-C), 115.01 (7-C), 114.46 (Ar3,5-C) 114.18 (Br3-C), 55.92 (OCH3). Calculated, %: C 69.12; H 4.16 Found, %: C 69.04; H, 4.21.

1-(5-chloro-1-benzofuran-2-yl)-3-(4-bromophenyl) prop-2-en-1-one (3b)

Yield: 88%, mp 198-199 oC IR spectrum, υ, cm-1: 1660 (C=O), 1611 (C=C). 1H NMR spectrum, δ, ppm: 8.27 s (1H, Br3-H), 8.00 s (1H, Br4-H), 7,92 d (1H, β-H, J=15.6 Hz), 7.88 d (2H, Ar3,5-H, J=8.4 Hz), 7,81 d (1H, α-H, J=15.6 Hz), 7.81 d (1H, Br6-H, J=8.6 Hz), 7.70 d (2H, Ar2,6-H, J=8.2 Hz), 7,60 d (1H, 7-H, J=8.8 Hz). 13C NMR spectrum, δC, ppm: 178.86 (C=O), 154.74 (Br2-C), 154.27 (8-C), 143.07 (β-C), 134.07 (Ar3,5-C), 132.47 (Ar2,6-C), 131.39 (Br5-C), 129.13 (9-C), 128.98 (Br6-C), 124.90 (Br4-C), 123.51 (Ar4-C), 122.94 (α-C), 115.04 (7-C), 114.53 (Br3-C). Calculated, %: C 56.43; H 2.77 Found, %: C 56.25; H, 2.68.

1-(5-chloro-1-benzofuran-2-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (3c)

Yield: 78%, mp 206-207 oC IR spectrum, υ, cm-1: 2850-2950 (C-H), 1649 (C=O), 1586 (C=C). 1H NMR spectrum, δ, ppm: 8,16 s (1H, Br3-H), 8.13 d (1H, β-H, J=15.6 Hz), 7.96 s (1H, Br4-H), 7.81 d (1H, Br6-H, J=8.8 Hz), 7.70 d (1H, α-H, J=15.6 Hz), 7.58 d (1H, 7-H, J=9.2 Hz), 7.52 s (1H, Ar6-H), 6,77 s (1H, Ar3-H), 3,93 s (3H, R1=OCH3), 3,90 s (3H, R3=OCH3), 3.85 s (3H, R4=OCH3). 13C NMR spectrum, δC, ppm: 178.89 (C=O), 155.26 (Ar2-C), 155.20 (Ar4-C), 154.03 (Br2-C), 153.94 (8-C), 143.56 (β-C), 138.87 (Ar5-C), 129.09 (Br5-C), 128.81 (9-C), 128.73 (Br6-C), 123.26 (Br4-C), 118.61 (α-C), 114.49 (7-C), 114.21 (Br3-C), 113.88 (Ar1-C), 111.26 (Ar6-C), 97.87 (Ar3-C), 56.89 (R1=OCH3), 56.79 (R3=OCH3), 56.35 (R4=OCH3). Calculated, %: C 64.39; H 4.56 Found, %: C 64.44; H 4.21.

1-(5-chloro-1-benzofuran-2-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (3d)

Yield: 58%, mp 185-186 oC IR spectrum, υ, cm-1: 2850-2950 (C-H), 1653 (C=O), 1586 (C=C). 1H NMR spectrum, δ, ppm: 8,12 s (1H, Br3-H), 8,07 d (1H, β-H, J=15.6 Hz), 7.96 s (1H, Br4-H), 7.93 d (1H, Ar6-H, J=8.4 Hz), 7.80 d (1H, Br6-H, J=8.4 Hz), 7.72 d (1H, α-H, J=15.6 Hz,), 7.58 d (1H, Ar5-H, J=8.8 Hz), 6.67 s (1H, Ar3-H), 3.93 s (3H, R1=OCH3), 3.87 s (3H, R3=OCH3). 13C NMR spectrum, δC, ppm: 179.02 (C=O), 164.04 (Ar4-C), 160.76 (Ar2-C), 155.16 (Br2-C), 154.06 (8-C), 139.18 (β-C), 130.86 (Ar6-C), 129.09 (Br5-C), 128.81 (9-C), 128.75 (Br6-C), 123.32 (Br4-C),

118.93 (α-C), 115.91 (7-C), 114.47 (Br3-C), 113.84 (Ar1-C), 107.05 (Ar5-C), 98.73 (Ar3-C), 56.36 (R1=OCH3), 56.08 (R3=OCH3). Calculated, %: C 66.53; H 4.38 Found, %: C 65.74; H 4.01.

1-(5-chloro-1-benzofuran-2-yl)-3-(4-methylphenyl) prop-2-en-1-one (3e)

Yield: 84%, mp 181-182 oC IR spectrum, υ, cm-1: 1653 (C=O), 1596 (C=C). 1H NMR spectrum, δ, ppm: 8.23 s (1H, Br3-H), 7.98 s (1H, Br4-H), 7.87-7.80 m (4H, Br6-H, β-H, α-H, Ar3,5-H), 7.59 d (1H, 7-H, J=8.8 Hz), 7.31 d (2H, Ar2,6-H, J=7.2 Hz), 2.37 s (3H, R3=CH3). 13C NMR spectrum, δC, ppm: 178.97 (C=O), 154.90 (Br2-C), 154.19 (8-C), 144.54 (β-C), 141.71 (Ar4-C), 132.06 (Ar1-C), 130.12 (Ar3,5-C), 129.58 (Ar2,6-C), 129.04 (Br5-C), 128.96 (9-C), 128.90 (Br6-C), 123.42 (Br4-C), 121.12 (α-C), 114.56 (7-C), 114.50 (Br3-C), 21.61 (R3=CH3). Calculated, %: C 72.81; H 4.38 Found, %: C 72.82; H 4.30.

1-(5-chloro-1-benzofuran-2-yl)-3-(3-nitrophenyl) prop-2-en-1-one (3f)

Yield: 55%, mp 194-195 oC IR spectrum, υ, cm-1: 1667 (C=O), 1614 (C=C). 1H NMR spectrum, δ, ppm: 8.81 s (1H, Ar2-H), 8.38 s (1H, Br3-H), 8.36 d (1H, Ar4-H, J=8.0 Hz), 8.31 d (1H, Ar6-H, J=8.0 Hz), 8,10 d (1H, β-H, J=16 Hz), 8.02 s (1H, Br4-H), 7.96 d (1H, α-H, J=16 Hz), 7.83 d (1H, Br6-H, J=8.4 Hz), 7.79 t (1H, Ar5-H, J=8.4 Hz, J=7.6 Hz), 7.62 d (1H, 7-H, J=8.8 Hz). 13C NMR spectrum, δC, ppm: 178.74 (C=O), 154.61 (Br2-C), 154.36 (8-C), 148.95 (Ar3-C), 141.87 (β-C), 136.66 (Ar6-C), 135.70 (Ar1-C), 130.94 (Ar5-C), 129.28 (Br5-C), 129.01 (9-C), 128.94 (Br6-C), 125.46 (Ar4-C), 124.86 (Ar2-C), 123.57 (Br4-C), 123.52 (α-C), 115.67 (7-C), 114.57 (Br3-C). Calculated, %: C 62.29; H 3.05 Found, %: C 62.04; H 2.98.

1-(5-chloro-1-benzofuran-2-yl)-3-(2-furyl) prop-2-en-1-one (3g)

Yield: 71%, mp 162-163 oC IR spectrum, υ, cm-1: 1657 (C=O), 1600 (C=C). 1H NMR spectrum, δ, ppm: 8.08 s (1H, Br3-H), 7.97 s (1H, Br4-H), 7.94 s (1H, Ar4-H), 7.82 d (1H, Br6-H, J=8.8 Hz), 7.67 d (1H, β-H, J=15.2 Hz), 7.59 d (1H, 7-H, J=8.8 Hz), 7.49 d (1H, α-H, J=15.2 Hz), 7.16 d (1H, Ar2-H, J=3.2 Hz), 6.73 dd (1H, Ar3-H, J=3.2 Hz, J=1.6 Hz). 13C NMR spectrum, δC, ppm: 178.61 (C=O), 154.77 (Br2-C), 154.13 (8-C), 153.11 (Ar1-C), 151.37 (Ar4-C), 147.22 (β-C), 130.83 (Br5-C), 129.07 (9-C), 128.92 (Br6-C), 123.36 (Br4-C), 118.74 (α-C), 118.59 (Ar2-C), 114.53 (7-C), 113.99 (Br3-C), 113.85 (Ar3-C). Calculated, %: C 66.03; H 3.30 Found, %: C 66.09; H 3.31.

1-(5-chloro-1-benzofuran-2-yl)-3-(2-thienyl) prop-2-en-1-one (3h)

Yield: 75%, mp 160-161 oC IR spectrum, υ, cm-1: 1660 (C=O), 1603 (C=C). 1H NMR spectrum, δ, ppm: 8.15 s (1H, Br3-H), 8.02 d (1H, β-H, J=15.6 Hz), 7.95 s (1H, Br4-H), 7.85 d (1H, Ar4-H, J=4.8 Hz), 7.81 d (1H, Br6-H, J=8.8 Hz), 7.74 d (1H, Ar2-H, J=2.8 Hz), 7.58 d (1H, 7-H, J=9.0 Hz), 7.51 d (1H, α-H, J=15.2 Hz), 7.23 dd (1H, Ar3-H, J=4.8 Hz, J=3.6 Hz). 13C NMR spectrum, δC, ppm: 178.54 (C=O), 154.73 (Br2-C), 154.18 (8-C), 139.91 (β-C), 137.27 (Ar1-C), 134.16 (Ar2-C), 131.67 (Ar4-C), 129.36 (Ar3-C), 129.06 (Br5-C), 128.93 (9-C), 128.90 (Br6-C), 123.37 (Br4-C), 120.32 (α-C), 114.51 (7-C), 114.27 (Br3-C). Calculated, %: C 62.36; H 3.12 Found, %: C 62.40; H 3.09.

1-(5-chloro-1-benzofuran-2-yl)-3-(1H-pyrrol-2-yl) prop-2-en-1-one (3i)

Yield: 48%, mp 233-234 oC IR spectrum, υ, cm-1: 3278 (N-H), 1642 (C=O), 1585 (C=C). 1H NMR spectrum, δ, ppm: 11,81 s (1H, NH), 7.99 s (1H, Br3-H), 7.82 s (1H, Br4-H), 7.78 d (1H, Br6-H, J=8.8 Hz), 7.69 d (1H, β-H, J=15.6 Hz), 7.57 d (1H, 7-H, J=8.8 Hz), 7.48 d (1H, α-H, J=15.6 Hz), 7.21 s (1H, Ar4-H), 6.82 s (1H, Ar2-H), 6.27 s (1H, Ar3-H). 13C NMR spectrum, δC, ppm: 178.56 (C=O), 155.60 (Br2-C), 153.92 (8-C), 134.75 (β-C), 129.46 (Ar1-C), 129.15 (Br5-C), 128.76 (9-C), 128.43 (Br6-C), 125.64 (Ar4-C), 123.24 (Br4-C), 117.58 (α-C), 114.65 (7-C), 114.33 (Br3-C), 112.37 (Ar2-C),

111.49 (Ar3-C). Calculated, %: C 66.27; H 3.68 Found, %: C 66.13; H 3.88.

Preparation of the Compounds

The chemical compounds used in this study are the newly synthesized molecules in the Firat University Faculty of Science Department of Chemistry. Ten different chemical compounds used in the study were dissolved using DMSO (dimethyl sulfoxide) in four different concentrations according to their molecular weights. The solutions were prepared in 4 different concentrations: 25 micromolar (μM), 50 micromolar (μM), 75 micromolar (μM), and 100 micromolar (μM).

Determination of Antimicrobial Activity

Microorganisms

In this study, gram positive (*Escherichia coli* ATCC 25322, *Klebsiella pneumoniae* ATCC700603) and gram negative (*Bacillus megaterium, Staphylococcus aureus*) bacteria were used. These microorganisms were obtained from the Fırat University Biology department microorganism collection. Bacteria were grown in Nutrient Broth and Mueller-Hinton Agar before the experimental study.

Agar Well Method

The agar well method was used to test the inhibition effects of chemical compounds on bacterial strains (31). The agar well method is a frequently used method to test plants, fungi, and chemical compounds whose antimicrobial effects are to be tested (32). Bacterial cultures within the broth are transferred to Müeller Hinton Agar then infused with the help of a loop and shaken well. It was placed in Petri dishes and kept for solidification of the medium completely. Petri dishes whose inoculation were completed were left to stand for 10-20 minutes to solidify at room temperature. The solidified Müeller Hinton Agar was used to create a well of the desired diameter, and agar piercing (corkborer) was used, and each time it was immersed in alcohol, it was sterilized by burning in a bunzen flame. With the sterilized corkborer, four wells were opened for each petri dish, except for negative and positive controls of the desired dimensions. Samples prepared from four different concentrations were placed in each well to be 100 μL. After inoculation, the petri dishes were left to incubate at 37 ˚ C for 24 hours. As a positive control; Clindamycin and amoxicillin were used, and 100% DMSO was added to the wells as a negative control. The diameters of the inhibition zones formed at the end of the incubation period were measured and recorded with the help of a ruler (33-36).

Determination of Minimum Inhibitory Concentration (MIC)

MIC value reported is described as the lowest concentration of the assayed antimicrobial agent that prevents the observable growth of the microorganism tested, and it is usually expressed in μg/mL or mg/L. There are several accepted criteria for dilution antimicrobial susceptibility testing of fastidious or non-fastidious bacteria, yeast, and filamentous fungi. Micro or macro-dilution is one of the most common research methods for antibacterial activity. This procedure is carried out on the liquid growth medium. The technique includes preparing a double dilution in tubes with a minimum volume of 2 mL (macrodilution), or with smaller amounts of the antibacterial agent, using 96 well microtitration plates (microdilution) (37). The sterile 96-well microtitrate plate, which had already contained 100 μL of nutrient broth, 100 μL of samples was applied to a nutrient Broth from rows 1-12, (end volume: 100 μL), which had been serially diluted and inoculated by 5 μL of different bacteria. The plate was incubated for 24 hours at 37 ˚C.

Determination of the Minimum Bactericidal Concentration (MBC)

The MBC is described as the smallest antimicrobial agent concentration necessary to kill 99.9 % of the final inoculum after 24hr. In which the microbial growth after incubation in the surface of non-selective agar plate can be determined by the subculturing from wells, resulting in a negative bacteria growth to determine the surviving number of cells (CFU/mL) after

24 h (37). The bottom on agar lowest broth with no growth in culture minimum concentration bactericidal concentration (MBC) considered (38). A part of the liquid (3 µl) in each MIC plates well, was taken for MBC determination, then incubated into the nutrient agar then for 24 hours at 37 °C. After subcultivation, MBC was taken as the lowest concentration, which showed no noticeable bacterial growth.

Results

In the present investigation, initially, *o*-alkylation of 5-chloro salicylaldehyde with chloroacetone in the presence of K_2CO_3 as an organic base furnished an *o*-alkylated salicyaldehyde derivative which subsequently generated enolate anion that underwent an intramolecular cyclocondensation reaction that afforded a new 1-(5-chloro-1-benzofuran-2-yl) ethanone **(1)**. A series of chloro-benzofuran substituted chalcones (3a-i) were prepared by the Claisen-Schmidt condensation of 1-(5-chloro-1-benzofuran-2-yl) ethanone and different aromatic aldehydes (Figure. 2). The structures of the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis.

In the FT-IR spectra of 1-(5-chloro-1-benzofuran-2-yl) ethanone, a C=O stretching vibration was observed 1671 cm^{-1}. The synthesized chalcones displayed two absorption bands in the range of 1614-1584 cm^{-1} and 1667-1642 cm^{-1}, which were assigned to the stretching vibrations of = C-H and C=O stretching, respectively; these were waving numbers that changed according to the structure of chalcones. The ^{1}H NMR spectrum of chalcones exhibited two characteristic doublets in the range of 8.11-7.48 ppm with the coupling constant (*J*) of approximately 15.6 Hz. The high value of the coupling constant established the *E*-geometry of the e-bond in the chalcones. A characteristic singlet in the range of 8.24-7.91 ppm due to 3-H belonging to the benzofuran ring confirmed the existence of the benzofuran ring. The ^{13}C NMR spectrum of the chalcones exhibited a peak of C-5 carbon atom-attached chlorine in the range 131.52-129.04 ppm. The signals due to the Cα and Cβ carbon atoms of the α, β-unsaturated carbonyl moiety of compounds were observed in the range of 123.52-117.58 ppm and 134.75-147.22, respectively. The peak in the region of about 178 ppm was attributed to the carbonyl carbon atom.

Antimicrobial Effects

In this study, the antimicrobial activity of ten different compounds on four different microorganisms was examined. The antibacterial effects of the obtained compounds were measured as a function of the diameter of the zone of inhibition (mm) in Table 1. DMSO was used as a negative control. The results were compared with standard drugs Amoxicillin and Clindamycin for antibacterial activity.

The highest effect of compound_1 at 100 µM concentration is against *K. pneumonia* and manifests itself with a 22 mm zone diameter. At 25 µM concentration, it was observed that among the four bacteria, it was most effective against *B. megaterium* with a 14 mm zone diameter (Figure. 3). The highest concentration (100 µM) of the compound_3a gives the most effect against *K.pneumonia* with a zone diameter of 25 mm, while the lowest concentration (25 µM) has the greatest effect on *E.coli*, *S.aureus* and *K.pneumonia*. 13 mm inhibition zone was detected against (Figure. 4). The highest concentration (100 µM) of the compound_3b is at its lowest concentration (25 µM), giving the 18mm zone diameter *on B.megaterium*, the highest effect is 13 mm on *E.coli*, *S.aureus,* and *B.megaterium*. It has been observed that it gives an inhibition zone (Figure. 5). 3c constitutes the highest effect at the highest concentration (100 µM) against *E.coli* and *K. pneumonia*, creating 17 mm zone diameter, while at the lowest concentration (25 µM), the maximum effect is against *E.coli* 16 mm zone. It was determined by showing the diameter (Figure. 6). The highest effect of the compound_3d at the highest concentration (100 µM) is against *E.coli* (21mm inhibition zone diameter) and at the lowest concentration (25 µM) is the highest effect against *E.coli* (16 mm zone diameter). that was determined (Figure. 7). The compound_3e produced the highest effect at the highest concentration (100 µM) as a 19 mm zone diameter against *B. megaterium* and the lowest concentration (25 µM) by giving the inhibition zone 15 mm in diameter against *B. megaterium* was observed (Figure. 8).

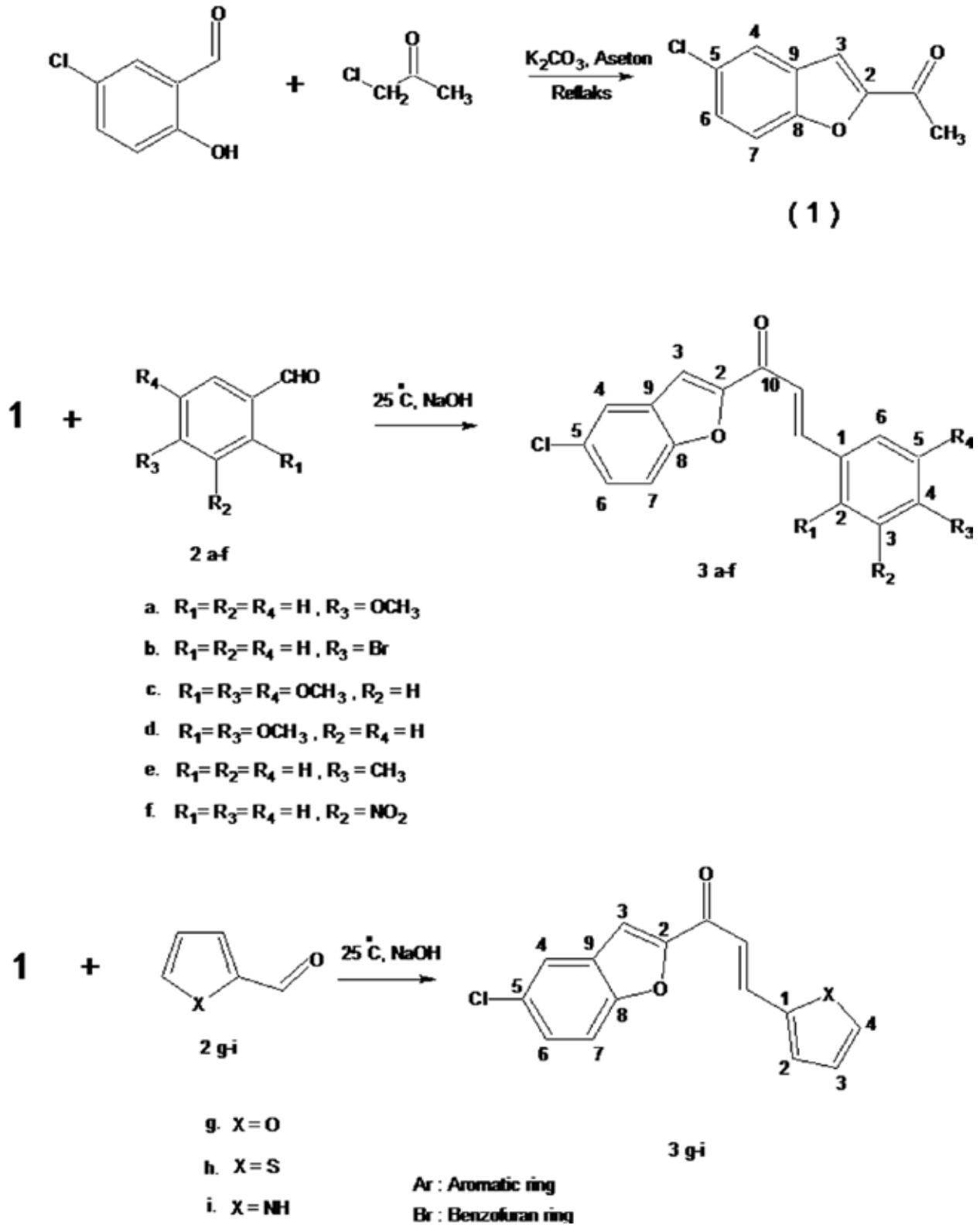

Figure 2. General synthesis of benzofuran chalcone derivatives

Table 1. Antimicrobial effects of chemical compounds on 4 different bacterial strains (Zone diameters as mm)

Compound numbers and Antibiotics		*E. coli*				*S. aureus*				*B. megaterium*				*K. pneumonia*			
		25 μM	**50** μM	**75** μM	**100** μM	**25** μM	**50** μM	**75** μM	**100** μM	**25** μM	**50** μM	**75** μM	**100** μM	**25** μM	**50** μM	**75** μM	**100** μM
1	**1**	12	14	15	17	13	14	14	17	14	15	16	17	12	13	17	22
2	**3a**	13	14	15	15	13	14	15	16	12	13	14	16	13	14	22	25
3	**3b**	13	14	16	17	13	13	15	12	13	14	15	18	11	12	14	15
4	**3c**	16	16	17	17	13	13	15	15	12	14	15	15	13	14	15	17
5	**3d**	16	18	19	21	11	17	17	15	12	13	15	16	13	15	16	17
6	**3e**	14	15	16	17	12	13	15	17	15	16	17	19	13	14	16	17
7	**3f**	13	14	15	17	13	14	15	16	13	14	15	16	12	14	17	22
8	**3g**	17	17	18	21	16	18	20	21	16	17	19	20	12	14	16	19
9	**3h**	17	18	20	20	11	12	14	16	13	14	15	16	14	15	19	20
10	**3i**	11	14	15	16	13	15	15	16	15	17	20	22	11	14	15	18
Amoxicilin		0				0				0				0			
Clindamycin		19				15				21				22			

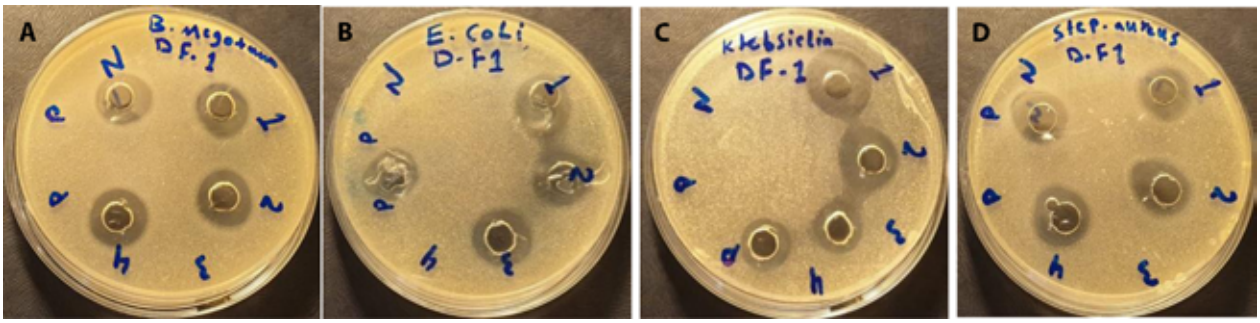

Figure 3. A. Inhibition zones of compound_1 (It was entitled as DF-1 on petri dish) against *B. megaterium*. **B.** Inhibition zones of compound_1 against *E.coli.* **C.** Inhibition zones of compound_1 against *K. pneumonia*. **D**. Inhibition zones of compound_1against *S. aureus.*

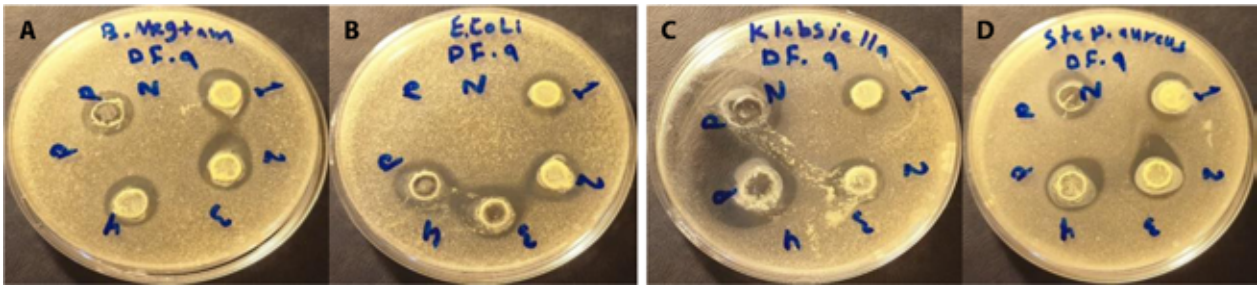

Figure 4. A Inhibition zones of compound_3a (It was entitled as DF-9 on petri dish) against *B. megaterium* **B.** Inhibition zones of the compound_3a against *E.coli* **C.** Inhibition zones of compound_3a against *K. pneumonia* **D.** Inhibition zones of compound_3a against *S. aureus*

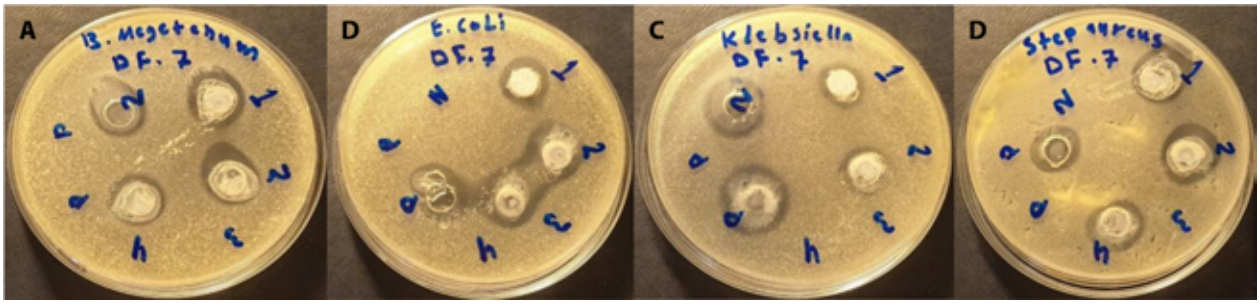

Figure 5. A. Inhibition zones of compound_3b(It was entitled as DF-7 on petri dish) against *B. megaterium* **B**. Inhibition zones of the compound_3b against *E.coli* **C.** Inhibition zones of compound_3b against *K. pneumonia* **D.** Inhibition zones of the compound_3b against *S. aureus*

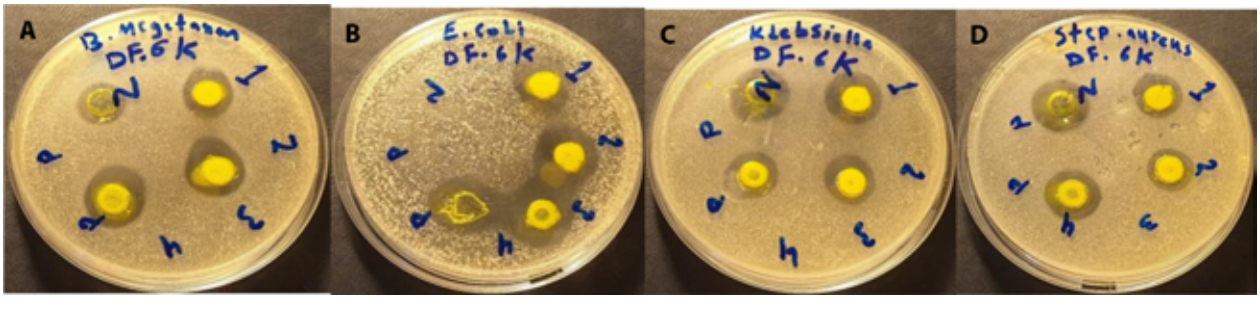

Figure 6. A. Inhibition zones of the compound_3c (It was entitled as DF-6k on petri dish) against *B. megaterium* **B.** Inhibition zones of the compound_3c against *E.coli* **C.** Inhibition zones of compound_3c against *K. pneumonia* **D.** Inhibition zones of compound_3c against *S. aureus*

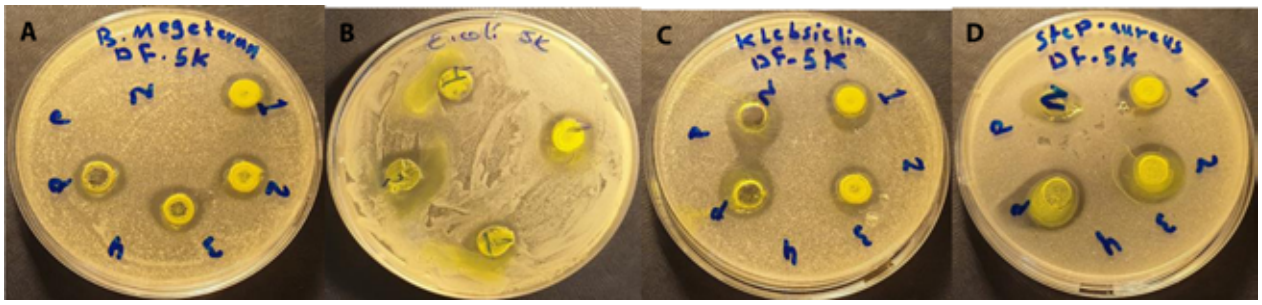

Figure 7. A Inhibition zones of the compound_3d (It was entitled as DF-5k on petri dish) against *B. megaterium* **B.** Inhibition zones of the compound_3d against *E.coli* **C.** Inhibition zones of compound_3d against *K. pneumonia* **D.** Inhibition zones of the compound_3d against *S. aureus*

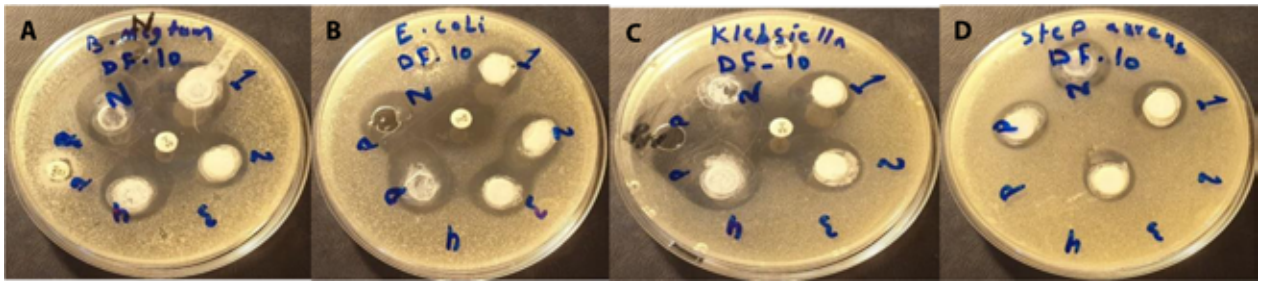

Figure 8. A Inhibition zones of compound_3e (It was entitled as DF-10 on petri dish) against *B. megaterium* **B.** Inhibition zones of compound_3e against *E.coli* **C**. Inhibition zones of compound_3e against *K. pneumonia* **D.** Inhibition zones of compound_3e against *S. aureus*

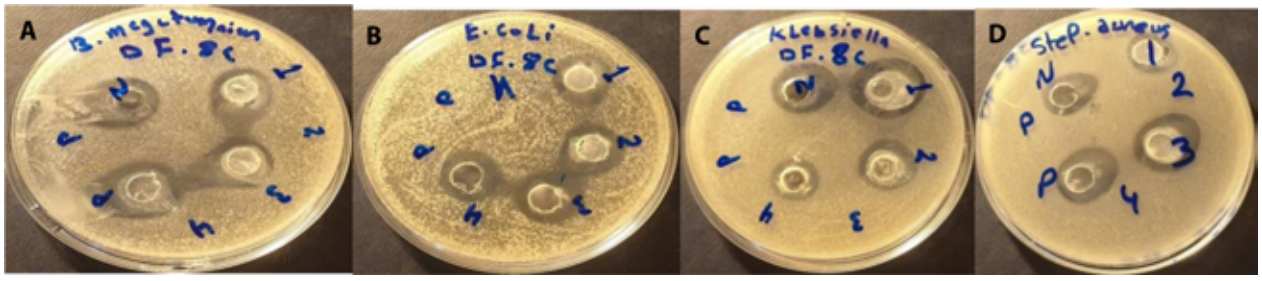

Figure 9. A. Inhibition zones of compound_3f (It was entitled as DF-8c on petri dish) against *B. megaterium* **B.** Inhibition zones of compound_3f against *E.coli* **C.** Inhibition zones of compound_3f against *K. pneumonia* **D.** Inhibition zones ofcompound_3f against *S. aureus*

The highest effect (100 μM) of the compound_3f gives the highest effect to *K.pneumonia* with a zone diameter of 22 mm, while the lowest concentration (25 μM) is the highest effect on *E.coli, S.aureus* and *B.megaterium*. 13 mm inhibition zone was found against (Figure. 9). The highest inhibitory effect of the compound_3g at the highest concentration (100 μM) was manifested against *E.coli* and *S. aureus* with a zone diameter of 21 mm. At the lowest concentration (25 μM), it was determined that it had the highest effect against *E.coli* with a zone diameter of 17 mm (Figure. 10). It was determined that the highest concentration (100 μM) of the compound_3h was the inhibitoriest effect against four different bacteria against *E.coli* and *K. pneumonia* (20 mm inhibition zone diameter). The lowest concentration (25 μM) was determined to have the inhibitoriest effect on *E.coli* (17 mm zone diameter) (Figure. 11). The highest concentration (100 μM) of the compound_3i was determined as the 22 mm zone diameter against *B.megaterium*, while the lowest

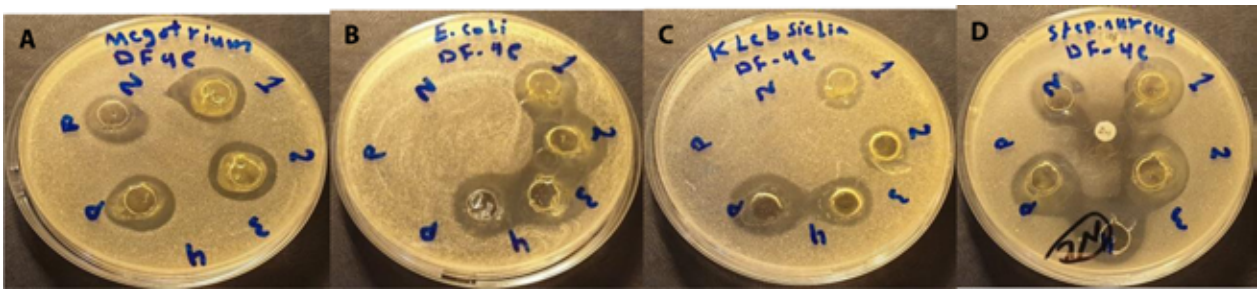

Figure 10. A. Obtained inhibition zones of compound_3g (It was entitled as DF-4e on petri dish) against *B. megaterium*. **B.** Inhibition zones of compound_3g against *E.coli.* **C.** Inhibition zones of compound_3g against *K. pneumonia* **D.**Inhibition zones of the compound_3g against *S. aureus*

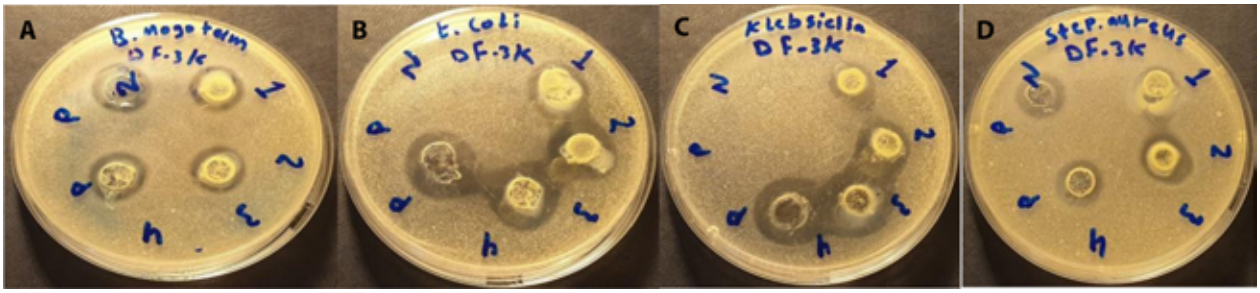

Figure 11. A. Inhibition zones of compound_3h (It was entitled as DF-3k on petri dish) against *B. megaterium*. **B.** Inhibition zones of compound_3h against *E.coli*. **C.** Inhibition zones of compound_3h against *K. pneumonia.* **D.** Inhibition zones of compound_3h against *S. aureus*

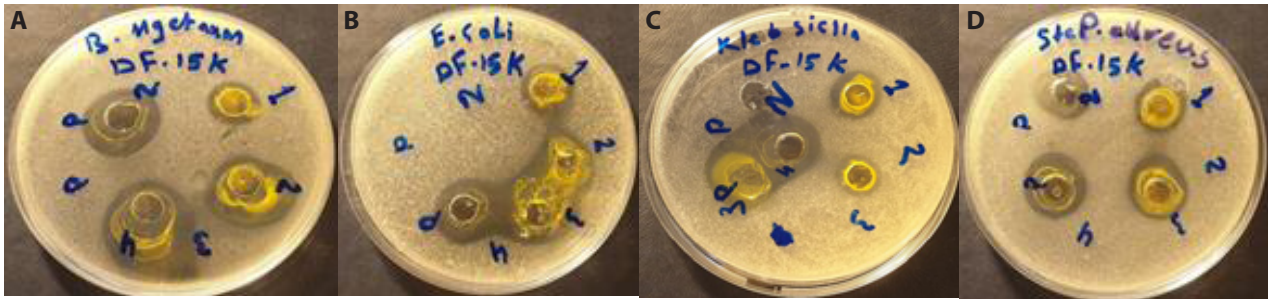

Figure 12. A. Inhibition zones of compound_3i (It was entitled as DF-15k on petri dish) against *B. megaterium* **B.** Inhibition zones of compound_3i against *E.coli* **C.** Inhibition zones of compound_3i against *K. pneumonia* **D.** Inhibition zones ofcompound_3i against *S. aureus*

concentration (25 μM) was given the greatest effect against *B.megaterium* with a 15 mm inhibition zone was detected (Figure. 12).

It was determined that *E.coli* formed 19 mm diameter zone against Clindamycin, *S.aureus* showed 15 mm diameter inhibition zone against Clindamycin. It was detected that *B.megaterium* showed 21mm inhibition zone against Clindamycin and *K.pneumonia* formed 22 mm diameter zone against Clindamycin. Abdel-Wahab et al. investigated the antimicrobial activity of some benzofuran chalcone derivatives on different bacterial and fungal strains. They reported that some chalcone derivative compounds showed significant antimicrobial activity on *E. coli*. These results are consistent with our results (30).

The minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the samples were determined against the four bacteria ranged from 1 to 1024 μg/ml. The MIC values varied depending on the test compounds. (Table 2)

Table 2. Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values were assessed according to their bioactivity.

Organism	*E. coli*		*K. pneumonia*		*S. aureus*		*B. megaterium*	
Sample	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	256	512	32	512	64	256	256	512
3a	256	512	128	256	128	256	16	256
3b	64	512	64	256	64	64	64	512
3c	128	256	128	512	128	256	32	256
3d	64	256	64	256	64	256	128	512
3e	16	256	32	256	32	256	32	256
3f	256	512	64	256	128	512	32	512
3g	64	256	64	128	128	512	128	256
3h	128	512	32	512	128	1024	512	512
3i	16	256	32	256	64	256	128	512

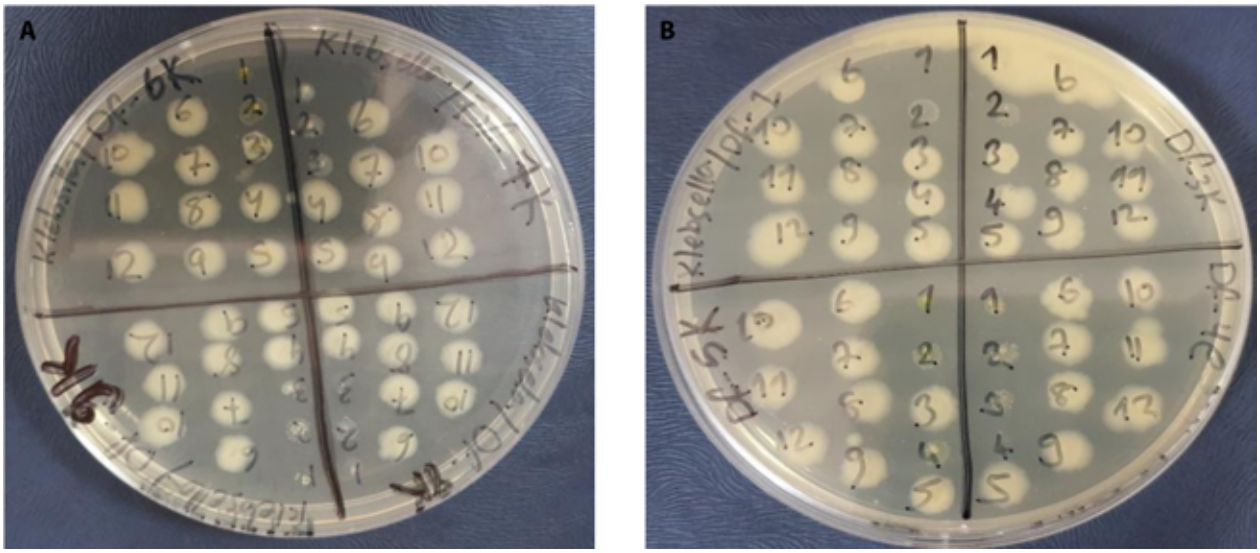

Figure 13. Effects of chemical samples were indicated in MBC test for *K. Pneumonia*

In the MIC technique, compound 3e and compound 3i have shown the highest ability to inhibit *E.coli* growth, while sample compound 1, compound 3f, and compound 3a have shown the lowest ability to inhibit *E.coli*. The highest MIC values (32 µg/ml) were obtained with compound 1, compound 3h, compound 3e, compound 3i against *K. pneumonia*, while the lowest value was determined with compound 3c and compound 3a. In the compression of MIC results between gram-positive bacteria (*S.aureus & B. megatarium*) the stronger effects were showed in (32 µg/ml) by *S.aureus* in the chemical samples compound 3e. While the lowest effect was seen with *B. megatarium* bacteria in the concentration (512 µg/ml) in the compound 3h. According to our results, Gram-negative bacteria have demonstrated a greater effect than Gram-positive bacteria in the MIC and MBC tests.

In the MBC results. *S. aureus* was found to be the most susceptible pathogen value of 64 mg/mL in the DF-7K samples while the same bacteria showed the highest defense against samples compounds 3h in 1024 mg/mL concentration. Besides that the MBC

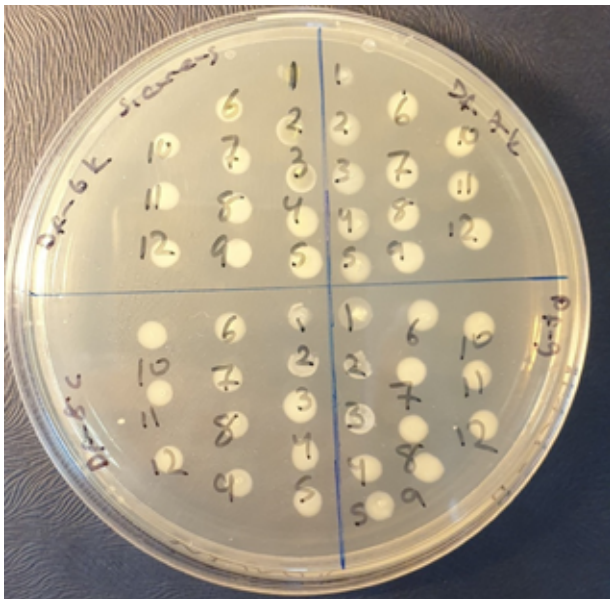

Figure 14. Effects of chemical samples were demonstrated test against *S. aureus* in MBC

effects of all samples against *E.coli, B. megaterium K. pneumonia* bacteria were ranged between 512-256 μg/mL. This result showed that in the opposite of MIC result, Gram-positive bacteria were stronger to inhibit compared to Gram-negative ones.

Discussion and Conclusion

In different study; the synthesis of 2-substituted pyrimidines by the reaction of benzofuran chalcones (3a-d) with urea, thiourea and guanidine hydrochloride was reported. Some of the compounds showed antimicrobial effect on *B. subtilis, E. coli* and *P. aeruginosa.* This study showed parallelism with our study (39). The range of benzofuran-based chalconoids 6a-v were projected and synthesized as new potential AChE inhibitors. The in vitro assay of synthesized compounds 6a-v showed had significant anti-AChE activity at

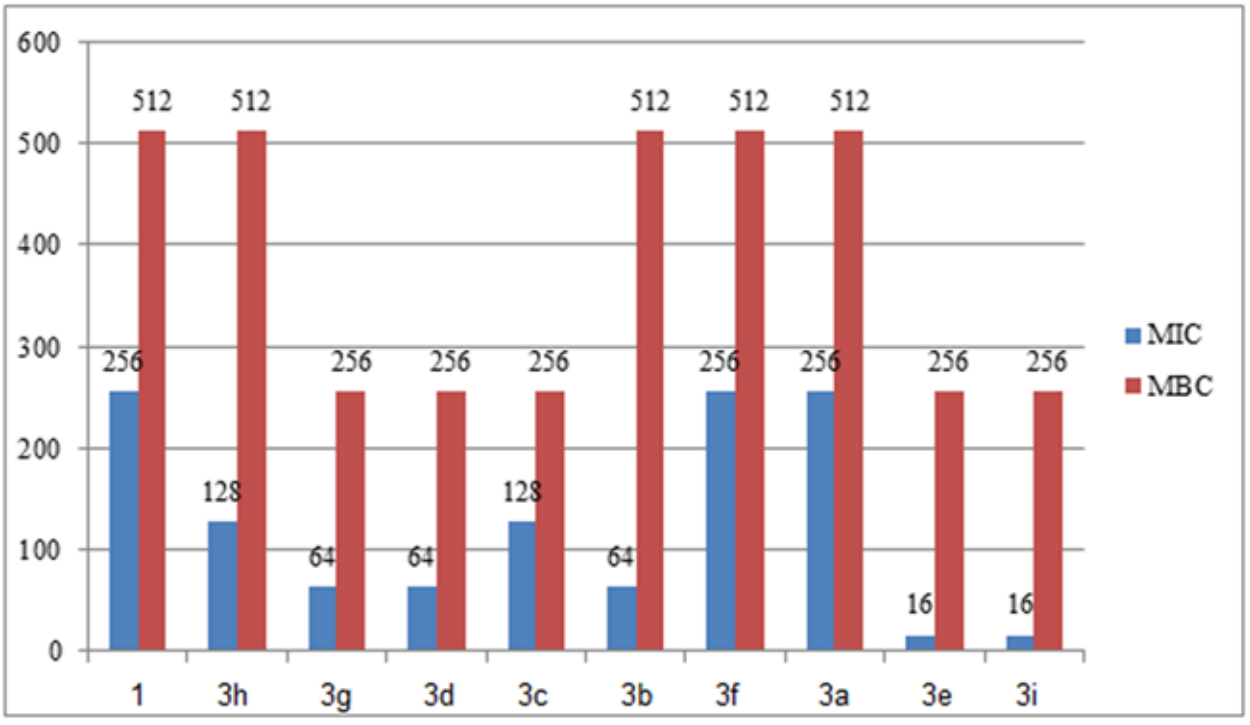

Figure 15. (MIC) effects of chemical samples were showed against *E. coli*

micromolar levels (40). 2-Acetylbenzofuran 1 on treatment with substituted aldehydes affords the corresponding chalcones 2a-c. The synthesized compounds were effected for their antimicrobial activities at 100 microg concentration. This compounds displayed antimicrobial activities (41). Fluorine containing heterocyclic system viz. 4-benzofuran-2-yl-6-phenyl-pyrimidin-2-ylamine has been synthesized and estimated for in vitro

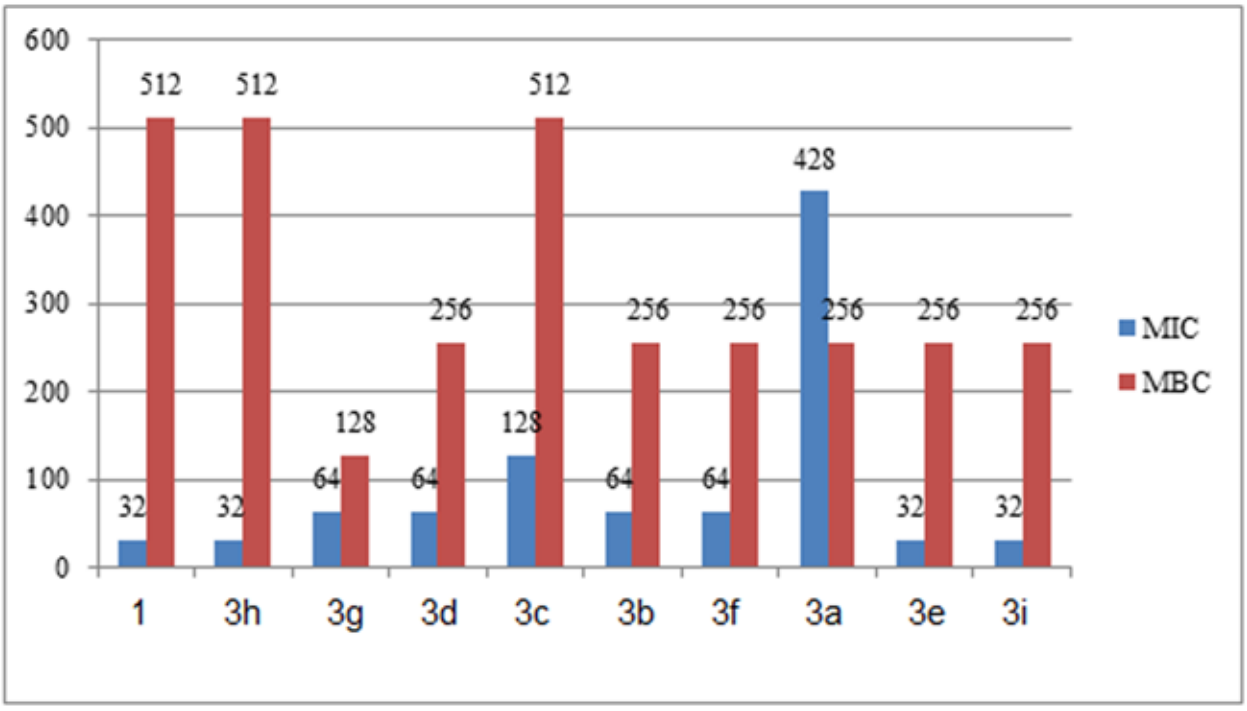

Figure 16. (MIC) effects of chemical samples were showed against K. pneumonia

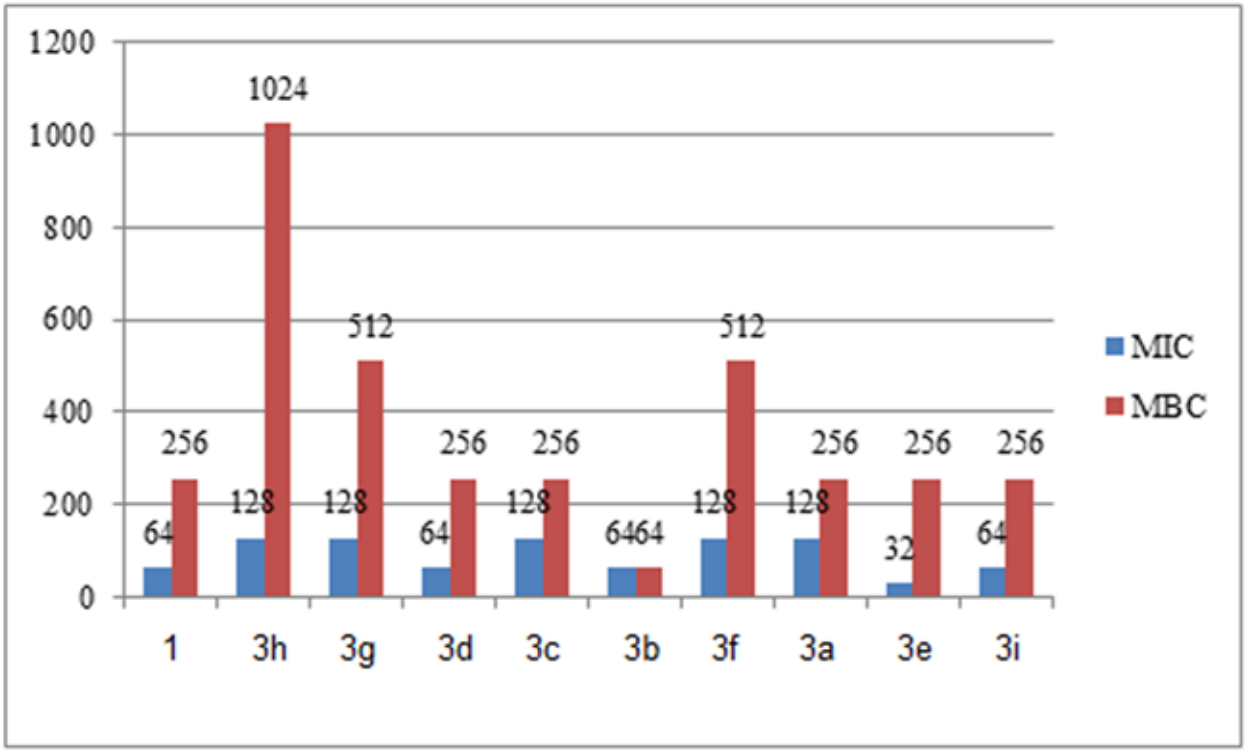

Figure 17. Effects of chemical samples were showed against S. aureus.

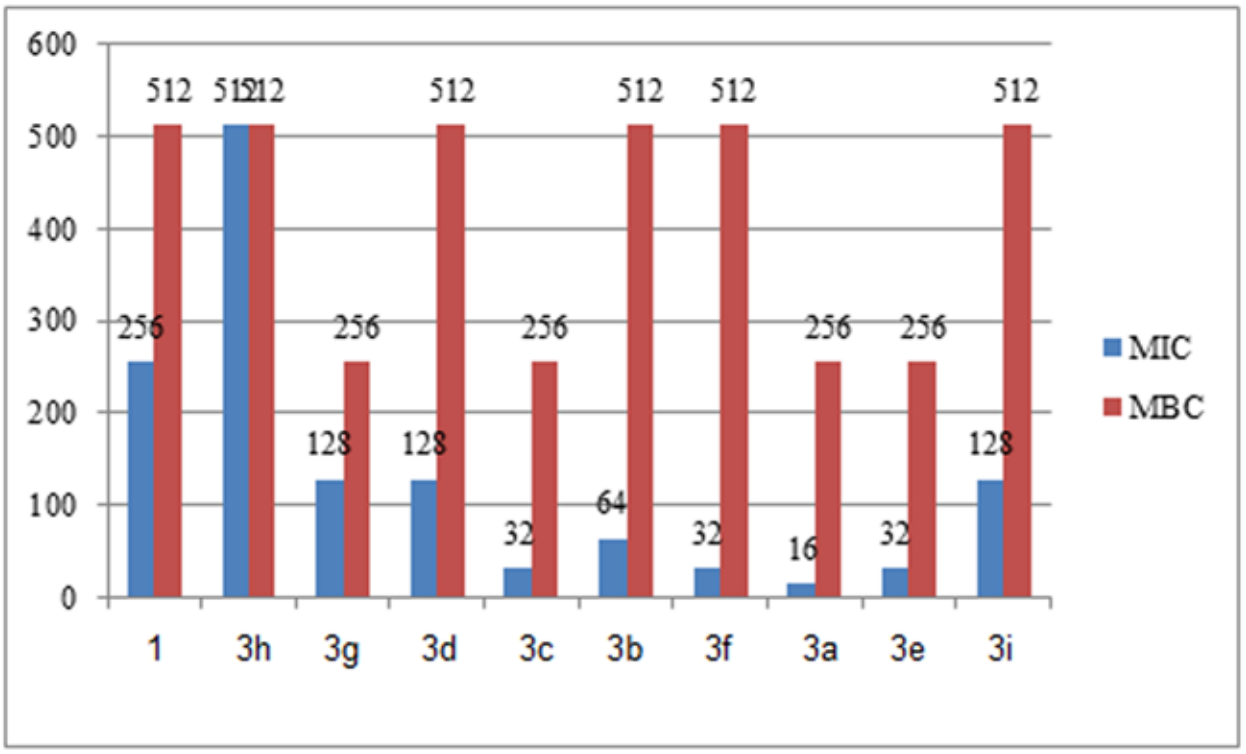

Figure 18. (MIC) effects of chemical samples were determined against B. megaterium

antibacterial and antifungal activities (42). Ten chalcones were tested against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. The unmodified compound that showed an inhibitory effect on all bacterial strains at minimum inhibitory concentrations ranging between 2 and 32 mg L (43). Chalcone has been synthesized as a new chalcone derivative bearing benzofuran moiety at 1 position. Such chalcone was used as a model dielectrophile applied to react with some nucleophiles such as 5-amino pyrazoles, 5-amino-1,2,4-triazole, 2-aminobenzimidazole, and 6-uraciles which synthesized compounds were estimated for their antimicrobial activities showed good activities (44).

The maximum zone diameters were determined as 21 mm at 100 μM concentration for compounds_ 3g and 3d while the smallest zone diameter was measured as 11 mm at 25 μM concentration compound_3i against *E.coli*. The highest inhibition zone was determined as 21 mm at 100 μM concentration for compound_3g while the smallest zone diameters were detected as 11 mm at 25 μM concentration for compounds_ 3h and 3d against *S.aureus*. The best inhibition zone diameter was measured as 22 mm at 100 μM concentration for compound_3i whereas the smallest inhibition zone was obtained as 12 mm at 25 μM concentration for compounds_3d, 3c, and 3a components against *B.megaterium*. The highest inhibition zone diameter was observed as 25 mm for compound_3a at 100 μM concentration, however, the smallest zone diameters were determined as 11 mm at 25 μM concentration for compounds_3b and 3i against *K.pneumonia.*

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