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Research Article

Synthesis, Characterization and Biological Activity of Some New Pyrimidine Derivatives

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Abstract

In this study, four derivatives of pyrimidine-2(1*H*)-thione were synthesized via the reaction between the compounds of α,β-unsaturated ketone and thiourea. The first step included a reaction between 1 mol of four different derivatives of primary amines and 1 mol of benzil. In the second step, the resulting compound was interacted with acetone to prepare α,β-unsaturated ketone. In the third step, the resulting compound was then interacted with thiourea to form the following compounds: 5,6-dihydropyrimidin-2 (1*H*)-thione. The IR, (^{1}H and ^{13}C) NMR and MS spectroscopies were used to confirm the structures of the prepared compounds and examine their antibacterial activities against (*Staphylococcus aureus* and *Escherichia coli*) by well diffusion method. It was found that the synthesized compounds have high activity against these two types of bacteria, especially compound P2 had the most inhibitory activity.

Keywords: amine, dicarbonyl, *Escherichia coli*, *Staphylococcus aureus*, thiourea

1. INTRODUCTION

Pyrimidine compounds are deemed as important heterocyclic organic compounds because they are used in the synthesis of biomolecules, say, nitrogenous bases which are deemed as the backbone of DNA and RNA composition [1]. Those two molecules are highly important in genetics where the sequence of such nitrogenous bases influences the inheritance of genetic traits [2][3]. Therefore, they contribute to composing nucleosides and nucleotides [4]. Many medicines and drugs contain pyrimidine molecules within their composition [5]. They are used to treat some types of inflammations and as an antivirus and antibacterial medicine [6][7]. Pyrimidine is found in three shapes, thymine, uracil, and cytosine [8]. Cytosine is involved in the composition of DNA and RNA. Thymine is used in the composition of DNA while uracil is used to compose RNA [9][10]. Pyrimidine is also involved in the composition of vitamin B6. Pyrimidine is an important compound in the field of organic chemistry, as many derivatives are prepared from it and are used in many fields, including medicine as antioxidants because it can eliminate free radicals, and in the industrial field as polymer antioxidants and plasticizers [11]. Due to its importance, there have been many methods used to prepare pyrimidine [12]. One of such method is the use of chalcone as a raw material of the reaction where pyrimidine is prepared through the reaction between chalcone with thiourea with the presence of alcoholic KOH with an abundant yield [13][14]. Based on the foregoing, a number of pyrimidine derivatives were prepared and their biological activities were examined as well.

2. MATERIALS AND METHODS

2.1. Materials

The chemicals used in the research were purchased from the companies located in Iran. Absolute Ethanol (abs. EtOH) 98%, glacial acetic acid (CH_3COOH) 99%, and acetone (CH_3COCH_3) 97% were purchased from Merck. Benzil 98%, sodium hydroxide (NaOH) 96%, urea (NH_2CONH_2) 98% and thiourea (NH_2CSNH_2) 98% were purchased from Sigma-Aldrich. The *p*-chloroaniline 95%, *p*-aminobenzoic acid 98%, *p*-nitroaniline 95%, and 1- naphthalamine 95.8% were purchased from Fluke. The prepared compounds were examined by the use of the following spectrometry: MS, NMR (integration ratios are used in ^{1}H-NMR to identify compounds), FTIR, and Shimadzu QP

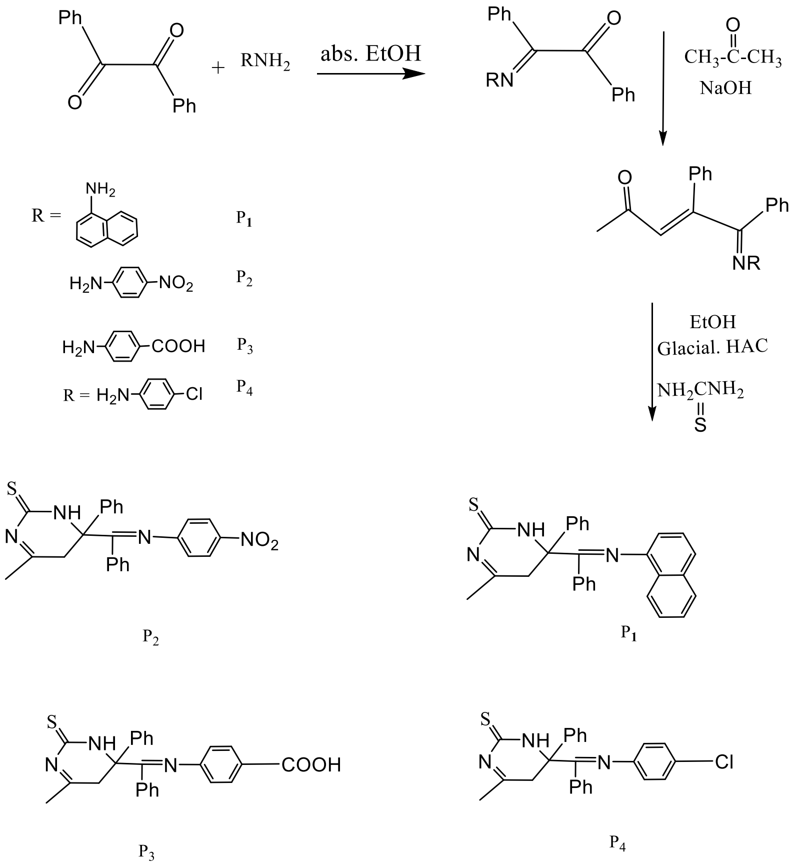

Figure 1. The synthesis of 5,6-dihydropyrimidin-2(1*H*)-thiones.

GC–MS. Melting point was measured by an electro-thermal IA device.

This research covers the synthesis of four pyrimidine compounds. Such preparation was made through three steps. In the first step, 1 mol of four different derivatives of primary amines have interacted with 1 mol of dicarbonyl compound (benzil). In the second step, the resulting compound was then interacted with α,β-unsaturated ketone. In the third step, the resulting compound was then interacted with thiourea in the presence of acetic acid as an acid catalyst to synthesize pyrimidine derivatives. The IR, (^{1}H and ^{13}C) NMR and MS spectrometries were used to confirm the composition of the prepared compounds. The antibacterial activity of such compounds against (*Staphylococcus aureus* and *Escherichia coli*) was studied and it has been confirmed that they do have high activity.

2.2. Methods

2.2.1. Imine Synthesis Method

Two solutions composed of benzil (0.016 mol) dissolved in absolute ethanol (35 mL) and primary amine (0.016 mol) dissolved in absolute ethanol (20 mL) were mixed under continuous stirring for 15 min under ambient temperature. A reflux was made then under 80 °C. Thin layer chromatography was used to observe the reaction. After the compound was filtered, ethanol was used for recrystallizing [15].

2.2.2. Chalcone Synthesis Method

A solution composed of amine (0.016 mole) dissolved in absolute ethanol (25 mL) was mixed with acetone (45 mL). Under continuous stirring for 30 min, 30 mL of NaOH 5% was added. After another 30 min of continuous stirring, the resulting compound was composed under ambient temperature. Thin-layer chromatography was used to observe the reaction. After the compound was filtered, ethanol was used for recrystallizing [16].

2.2.3. Pyrimidine Synthesis Method

A compound composed of chalcone (0.01 mol) dissolved in ethanol (25 mL) was mixed with another compound composed of thiourea (0.01 mol) dissolved in water (25 mL). The mixing of such compounds was made under continuous stirring. Three drops of acetic acid as a catalyst were added. After 15 min of continuous stirring, a reflux was made under 80 °C. Thin-layer chromatography was used to observe the reaction. After the compound was filtered, ethanol was used for recrystallizing [1].

Figure 1 shows the equations for the synthesis of new compounds. Figure 2 shows the mechanism for the synthesis of Schiff base compounds while Figure 3 shows the mechanism for the synthesis of chalcones. Figure 4 shows the mechanism for the synthesis of pyrimidine compounds. Table 1 shows the physical properties of the synthesized compounds. Spectroscopic methods confirmed the correctness of the prepared structures.

Compound P1 was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopies as shown in Figures 5 (a), 6(a), 7(a) and 8(a), respectively. Compound P2 was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopies as shown in Figures 5 (b), 6(b), 7(b), and 8(b), respectively. Compound P3 was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopies as shown in Figures 5(c), 6(c), 7(c), and 8(c), respectively. Compound P4 was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopies as shown in Figures 5(d), 6(d), 7(d) and 8(d), respectively.

2.2.4. Anti-Bacterial Study

Two types of bacteria, *E. coli* and *S. aureus* were used to examine the antibacterial activities of the four compounds synthesized subject to this research under aerobic condition [17][18]. Well-diffusion method was used along with DMSO solvent. Overnight cultures were used for the four synthesized compounds to examine their antibacterial activities against the above types of bacteria [19]. Such cultures were incubated for 24 h. A saline solution was used for the purpose of dilution and to maintain that the test diffusion

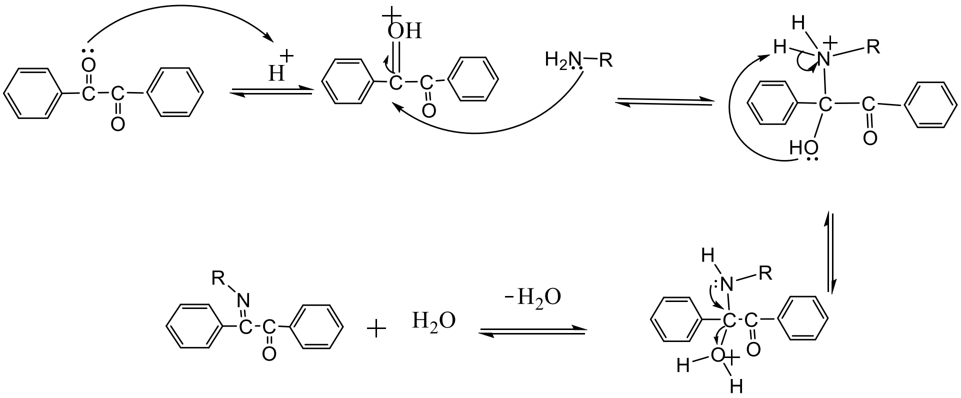

Figure 2. Mechanism for the synthesis of Schiff base compounds.

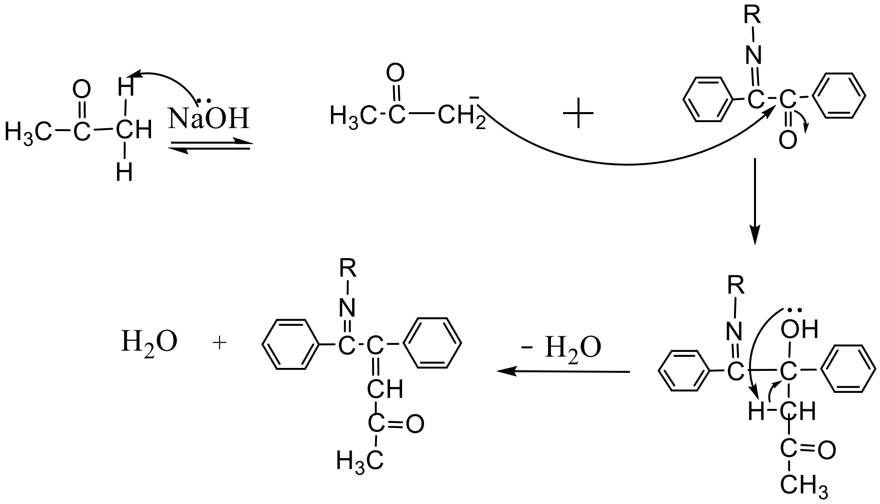

Figure 3. Mechanism for the synthesis of chalcones.

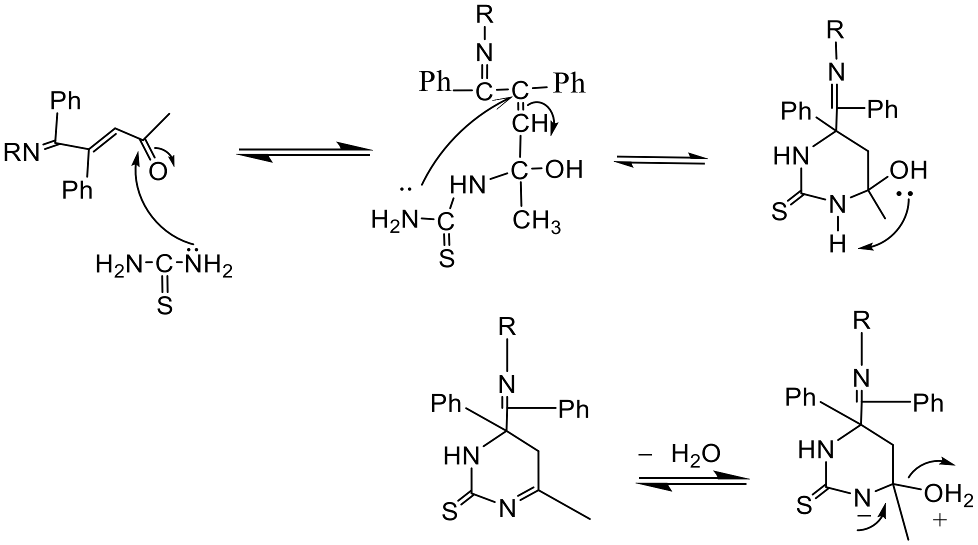

Figure 4. Mechanism for the synthesis of pyrimidines.

Table 1. Physical and chemical properties of synthesized 5,6-dihydropyrimidin-2(1*H*)-thiones.

Compounds	Melting point (°C)	Yield %	Solvents (v/v)	Rf	Color
P1	133–134	80	3:7 – hexane:ethyl acetate	0.40	Brown
P2	142–144	76	5:5 – hexane:ethyl acetate	0.34	Brown
P3	135–136	69	5:5 – hexane:ethyl acetate	0.50	Orange
P4	137–140	60	6:4 – hexane:ethyl acetate	0.35	Yellow

applies to 10^8 CFU/mL, (turbidity used McFarland $BaSO_4$ Standard 0.5).

2.2.5. Diffusion Method

Agar containing jelly with 7 mL width and 7 mL space length was used [20]. The bacterial vaccine was then uniformly spread on a sterilized Petri dish by the use of sterilized cotton. The compounds were prepared out of the four compounds with 100 mg/mL concentration. 50 mL of each compound was used and spread on bacteria–applied Petri dishes. Such dishes were then incubated for twenty-four hours at 36±1 °C. After the incubation period was over, inhibition diameters were measured as shown in Figure 9 and listed in Table 2.

3. RESULTS AND DISCUSSIONS

3.1. 4-methyl-6-((naphthalen-1-ylimino)(phenyl)methyl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-thione (P1)

Brown powder; Yield 80%; m. p, 133–134 °C; TLC solvents (3:7 – hexane:ethyl acetate) (Rf = 0.40); ^{1}H-NMR, [DMSO, 300 MHz, δ ppm]: 2.07 (s, 2H, CH_2), 1.99 (s, 3H, CH_3), 9.25 (H, NH), 9.14–6.12 (m, 17 H, Ar); ^{13}C-NMR, [DMSO, 300 MHz, δ ppm]: 40.65, 57.43, 59.00, 122.87, 122.90, 122.92, 122.95, 124.93, 124.99, 125.00, 125.45, 125.47, 125.88, 126.11, 126.53, 126.57, 126.87, 127.99, 129.33, 131.16, 144.73, 145.79, 164.00, 166.90; FT-IR, ν cm^{-1}: 1548 (C=C), 1600 (C=N), 748 (C=S), 3301 (N–H), (3001 C–H aromatic), 2809–2880 (CH aliphatic); m/z, 433 (M^+).

3.2. 4-methyl-6-(((4-nitrophenyl)imino)(phenyl)methyl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-thione (P2)

Brown powder, Yield 76%; m. p, 142–144 °C; TLC solvents (5:5 – hexane:ethyl acetate) (Rf = 0.34); ^{1}H-NMR, [DMSO, 300 MHz, δ ppm]: 2.01 (s, 2H, CH_2), 1.94 (s, 3H, CH_3), 8.31 (H, NH), 8.29 –6.33 (m, 14 H, Ar); ^{13}C NMR, [DMSO, 300 MHz, δ ppm]: 39.67, 40. 09, 124.09, 124.62, 124.80, 127.02, 127.19, 127.41, 127.81, 128.76, 128.91, 129.07, 130.00, 130.08, 132.66, 136.07, 160.33, 195.33; FT-IR, *ν* cm^{-1}: 1550 (C=C), 1557 (C=N), 770 (C=S), 3360 (N–H), 3161 (C–H aromatic), 2876–2899 (C–H aliphatic), 1349–1500 (NO_2); m/

z, 429 (M^+).

3.3. 4-(((6-methyl-4-phenyl-2-thioxo-2,3,4,5-tetrahydropyrimidin-4-yl)(phenyl)methylene)amino) benzoic acid (P3)

Orange powder; Yield 69%; m. p, 135–136 °C; TLC solvents (5:5 – hexane:ethyl acetate) (Rf = 0.50); ^{1}H-NMR, [DMSO, 300 MHz, δ ppm]: 3.45 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 9.26 (H, NH), 9.15 –6.13 (m, aromatic protons), 11.80 (s, OH); ^{13}C-NMR, [DMSO, 300 MHz, δ ppm]: 39.08, 39.89, 40.86, 60.09, 112.76, 113.11, 114.85, 114.32, 114.65, 118.65, 118.89, 136.87, 137.76, 137.88, 150.32, 160.55, 160.64 165.60, 165.99; FT-IR, *v* cm^{-1}: 1540 (C=C), 1557 (C=N), 750 (C=S), 3320 (N–H), 3321 (OH), 3101 (C–H aromatic), 2964–

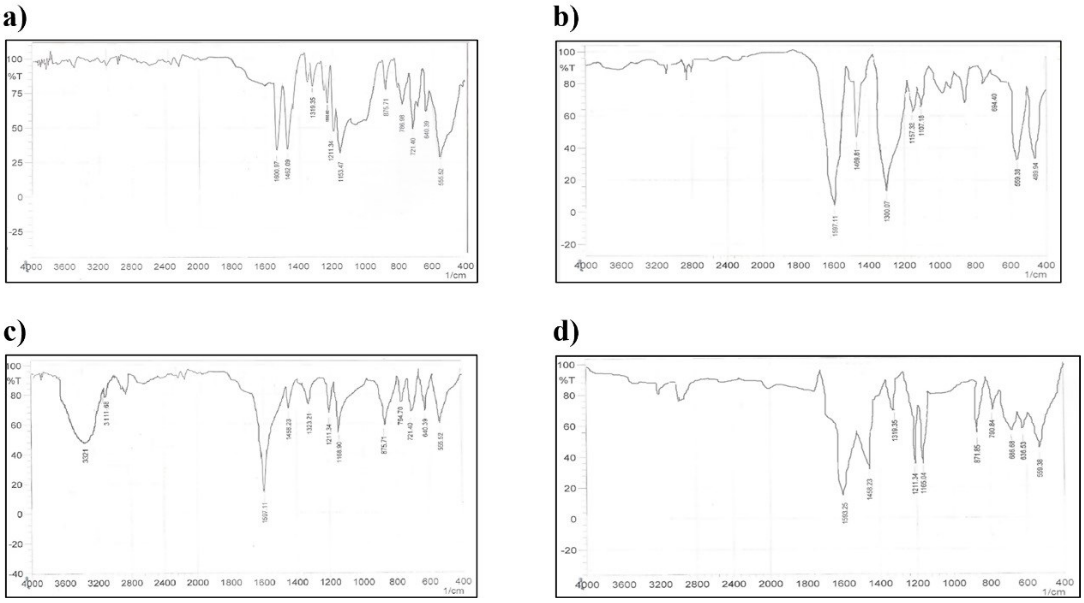

Figure 5. FT-IR spectra of a) P1, b) P2, c) P3, and d) for P4.

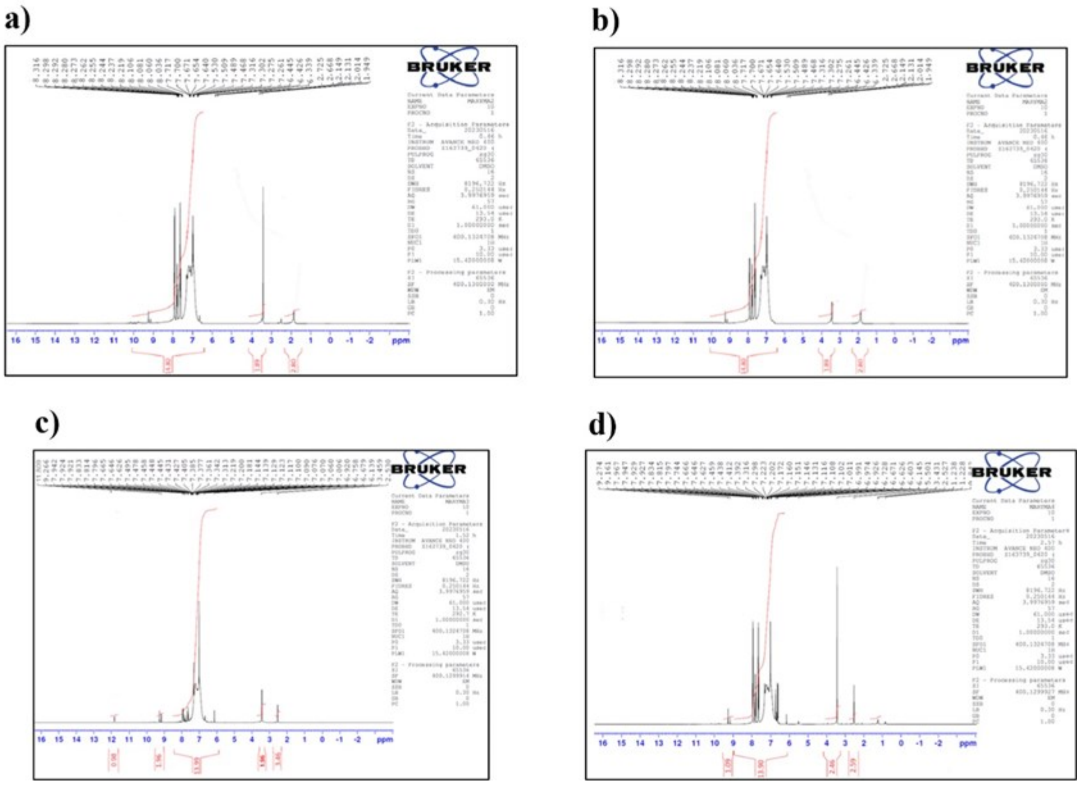

Figure 6. ^{1}H-NMR spectra of a) P1, b) P2, c) P3, and d) P4.

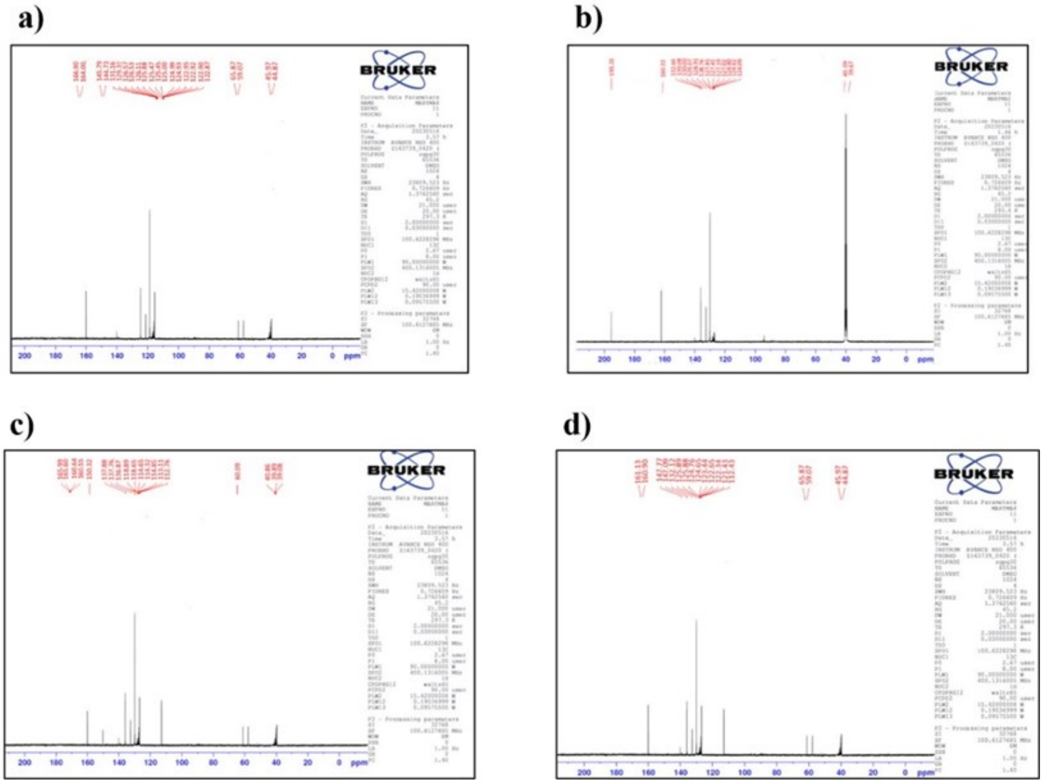

Figure 7. ^{13}C-NMR spectra of a) P1, b) P2, c) P3 and d) P4.

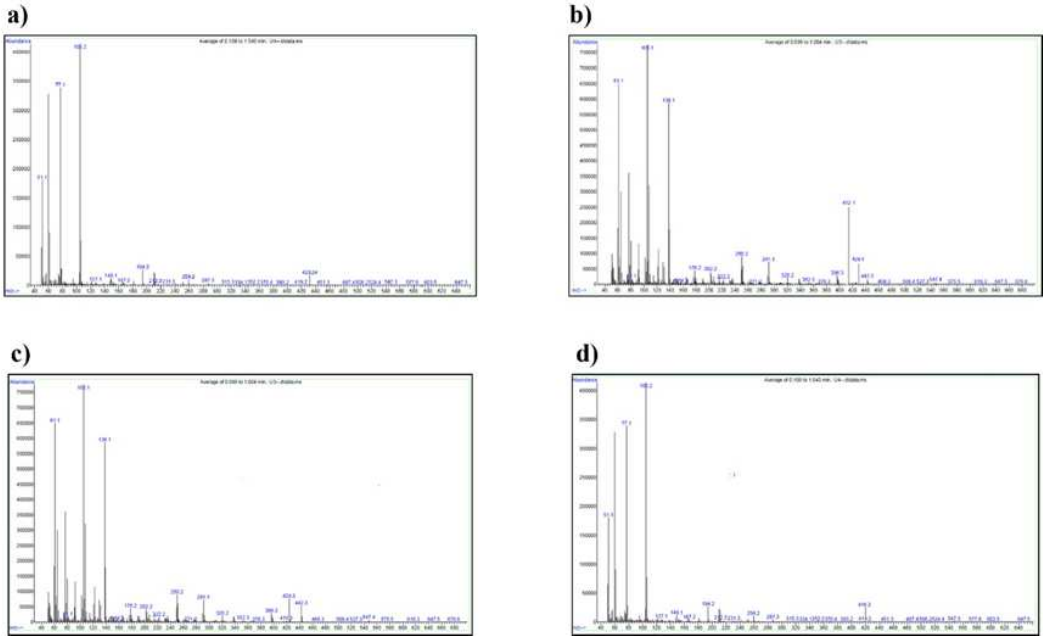

Figure 8. Mass spectra of a) P1, b) P2, c) P3, and d) P4.

2989 (C–H aliphatic); m/z, 428 (M$^+$).

3.4. 6-(((p-chlorophenyl) imino) (phenyl)methyl)-4-methyl-6-phenyl-5,6-dihydropyrimidin-2(1H)-thione (P4)

Yellow powder; Yield 60%; m. p, 137–140 °C; TLC solvents (5:5 – hexane:ethyl acetate) (Rf = 0.50);^{1}H-NMR, [DMSO, 300 MHz, δ ppm]: 2.62 (s, 2H, CH_2), 1.22 (s, 3H, CH_3), 9.27 (H, NH), 9.19–5.50 (m, 14 H, Ar); ^{13}C NMR, [DMSO, 300 MHz, δ ppm]: 44.87, 45.97, 59.07, 65.87, 112.43, 121.43, 122.34, 122.65, 123.44, 124.65, 124.76, 125.88, 125.89, 132.12, 147.09, 147.77, 160.90, 161.13; FT-IR, v cm^{-1}: 1553(C=C), 1600 (C=N), 771 (C=S), 3332 (N–H), 3001 (C–H aromatic), 2890–2945 (C–H aliphatic), 670 (C–Cl); m/z, 418 (M^+).

All FT-IR spectra of the prepared compounds demonstrated an absorbance band of stretching vibration of C=S bond in addition to another group of vibrational absorptions of aliphatic C–H, C=N, C=C, N–H, and C–H aromatic bonds. P2 compound is characterized by the demonstration of two stretching vibration bands in the regions of 1500 and 1349 cm^{-1} for asymmetrical and symmetrical NO_2 group. P3 compound is characterized by the demonstration of the stretching vibration band belonging to OH group in the 3400 cm^{-1} region while P4 compound spectrum is characterized by the demonstration of the absorbance band of stretching vibration in 670 cm^{-1} regions for C–Cl.

Integration ratios are used in ^{1}HNMR to identify compounds. Spectrum of compound P1 showed the presence of the naphthyl protons, while compound P2 showed a higher shift due to the presence of the electron-withdrawing NO_2 group, which causes un-shielding. The spectrum of compound P3, which contains the COOH group, showed the presence of the OH group, which was shifted to position 11.80 ppm. The compound P4 was distinguished by the presence of Cl as a substitute, and its effect was clearly evident in the spectrum, as it caused a higher shift of the protons of the phenyl ring.

For ^{13}C-NMR spectra, each compound has signals indicating the correct number of carbon atoms. Carbon atoms with similar properties under the surrounding conditions all show a single signal. This proves the validity of the structures of the synthesized compounds. For the MS spectra, the spectra revealed the parent ion of each compound, as well as molecular fragments resulting from the fragmentation. This confirms the structure of synthesized compounds.

3.5. Antibacterial Studies

It was found that the synthesized compounds had a significant effect against the two types of bacteria (*S. aureus* and *E. coli*). All Petri dishes were incubated under aerobic conditions at 37 °C for 24 h. DMSO was used as a solvent for antibacterial testing due to its ability to dissolve a wide range of organic compounds. The final DMSO concentration in wells was below 1%, a level shown not to interfere with bacterial growth. Among the synthesized compounds, P2 exhibited the highest antibacterial activity. The diameters of inhibition was 25 mm against the two types of bacteria, which may be attributed to the presence of the nitro group at the para position. The electron-withdrawing nature of the nitro group may enhance the compound's ability to penetrate bacterial cell walls and interact with intracellular targets. This structural feature likely contributes to the improved inhibitory zones observed.

Compound P1 contains an aromatic substituent (naphthyl group) and has no polarity, which means that it dissolves in the lipids of the bacterial cell membrane Therefore, inhibition is equal as the diameters of inhibition were 23 and 25 mm against *S. aureus* and *E. coli*, respectively. Then, compound P4 ranked third in terms of its ability to inhibit, as the diameters of inhibition were 23 and 22 mm against *S. aureus* and *E. coli*, respectively. Chloride

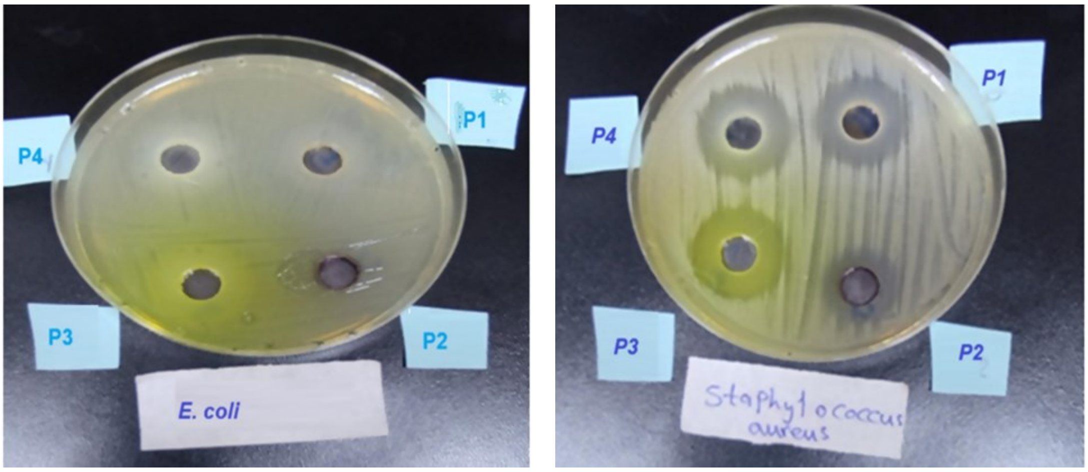

Figure 9. Photographic images of bacterial inhibition zones.

Table 2. Bacterial inhibition diameters.

Compounds	Concentration (mg/mL)	Inhibition zone (mm)	
		Staphylococcus aureus	*E. coli*
P1	100	23	25
P2	100	25	25
P3	100	18	0
P4	100	23	22

is small in size and therefore can penetrate the lipid membrane and affect *S. aureus* bacteria. Chloride has little polarity, but this enables it to interact with *E. coli* bacteria.

The least effective against bacteria was compound P3, as the diameters of inhibition were 18 mm against *S. aureus* and had no inhibitory effect against *E. coli*. Compound P3 forms hydrogen bonds due to the presence of the COOH group, and the molecules of this compound may be linked together in a specific spatial configuration, which makes them ineffective in the case of *E. coli.* It can dissolve in the lipid membrane and therefore affects *S. aureus*. The action of the prepared compounds is similar to the action of ampicillin in its activity against bacteria, as ampicillin interferes with the process of building the bacterial cell membrane, and this is indicated by the difference in effectiveness with the change of the substitutes. When comparing the activity of P2 (25 mm inhibition zone) we found it similar to pyrimidine derivatives reported in the literature.

4. CONCLUSIONS

This research covers the synthesis of four pyrimidine-2(*H*)-thiones. A Schiff base was synthesized by the reaction between the primary amine and the carbonyl groups of benzil compounds. Then, the chalcone was synthesized by the reaction between the prepared compound and acetone to produce an α,β-unsaturated ketone, which was then reacted with thiourea to form the final product 5,6-dihydropyrimidine-2(*H*)-thione. The spectroscopic methods confirmed the correct structures of the synthesized compounds. The antibacterial activity of these compounds was studied, and it was observed that they possessed high antibacterial activity. Compound P2 was found to be the most effective, with activity equal to that of *S. aureus and E. coli*, and an inhibition diameter of 25 mm, likely due to the presence of an electron-withdrawing nitro group. These results suggest that structural modifications on the aryl moiety can significantly impact biological activity, warranting further optimization and *in vivo* evaluation.

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Conflicts of Interest

There is no conflict of interest. There is no financial funding from any side as the support was self-supporting.

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