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FULL PAPER

Biological activity of new heterocyclic compounds derived from chalcone

Sura Sadiq Obaid* |Nour Abd Alrazzak Abd Allatif

Department of Chemistry, College of Science for Women, University of Babylon, Hilla, Iraq

***Corresponding Author:**
Sura Sadiq Obaid
E-mail: sura.alsultany11@gmail.com
Tel.: +9647811254605

In this work, chalcones were synthesized by condensing ketone containing azo dye with aromatic aldehyde derivative (Vanillin) at room temperature and in a diluted ethanolic potassium hydroxide solution. The subsequent reaction of chalcone with urea, thiourea, hydrazine hydrate, and hydroxylamine hydrochloride led to the synthesis of novel heterocyclic derivatives including oxazine, thiazine, pyrazol, and isoxazole, respectively. After that, the effect of these compounds was studied on gram-positive (*Staphylococcus Epiderimidis*, *pseudomonas*, and gram-negative (*Klebsiella pneumoniae and E.Coli*) bacteria. The (FTIR, ^{1}H-NMR, and ^{13}C-NMR) spectroscopies were used to characterize each of the synthesized compounds.

KEYWORDS

Synthesis; azo; chalcone; antibacterial; characterization.

Introduction

Due to the presence of chromophore group (N = N-) connected with aromatic or heterocyclic systems, azo compounds are distinguished by their exceptional coloring capabilities [1].

Heterocyclic chemistry research includes almost half of the organic chemistry research through the world. The field of medicine relies heavily on a large number of bioactive organic molecules with heterocyclic frameworks. It is frequently stated that heterocycles have the architecture of the majority of pharmaceutical and natural chemicals that often contains either an atom of nitrogen, sulfur, or both [2].

Chalcone is a substance containing two aromatic rings joined by a system of three, unsaturated carbonyl groups. It is also known as 1,3-diphenyl-2-propenee-1-one, (Figure 1)[3].

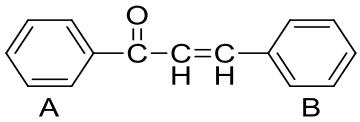


FIGURE 1

Chalcones are phenyl-styryl ketones that differ from other types of ketones by the position of their reactive ketoethylenic group (C-COCH) in the Ar(A)-CO-CH= CH-Ar(B) structure [4].

In the 21st century, research on medicinal chemistry has focused heavily on natural and synthetic chalcone due to their extensive pharmacological potential, including actions and characteristics, for instance, antibiotics [5-6] anti-inflammatory [7] antioxidant [8], anti-platelet [9], anti-cholinergic [10], and anti-inflammatory medicines that treat diabetes [11,12], cancer [13], viruses [14], and leishmaniasis [15].

Chalcone had been used as intermediates for the preparation of mixtures having therapeutic value, due to the pyrimidine ring system's significant pharmacological action, pyrimidines are important in both chemical and biological processes. The NH_2CONH_2 group acts as an ant thyroid compound, with the same actions and uses as thiouracil [16].

The payroll ring is a major structural motif included in various pharmaceutically active molecules. Pyrazole has essential biological effects, including those for osteoporosis, postmenopausal symptoms, and inflammation [17].

Aim of work

This study was done to synthesize and characterize new azo and chalcone compounds, synthesize new (five and six) members heterocyclic compounds, and to study the biological activity of some of the prepared compounds.

Experimental

Chemicals

All chemical compounds that have been supplied are of high purity (CDH,Merk): *p*-amino benzoic acid, 2,4-dihydroxy acetophenone, 3-methoxy-4-hydroxy benzaldehyde, urea, thiourea, hydrazine hydrate, and hydroxylamine hydrochloride.

Instruments

The infrared spectra were measured using a KBr disk and Fourier transform infrared spectrophotometer (FT-IR–8400S) from Shimadzo. The Oxford 400 Magnet and NMR Innova 5 Concole were used to measure ^{1}H-NMR spectra and ^{13}C-NMR in DMSO as a solvent. Melting pints were verified by hot stage SMP30 melting point apparatus. The biological activity was performed by Microbiology Unite, Babylon Hospital.

Methods

Synthesis of 4-(5-acetyl-2,4-dihydroxyphenyl)diazenyl) benzoic acid [S1]

P-aminobenzoicacid (1.37g, 0.01 mole) was dissolved in 17 mL of distilled water and 3 mL of HCl at a temperature of (0 to 5 °C). The solution was then added dropwise for 15 minutes using 0.01 mole (0.69 g) of $NaNO_2$ dissolved in 10 mL of distilled water. By combining (0.01 mole, 1.520 g) of 2,4-dihydroxy acetophenone in ethanol with 1 g of sodium hydroxide in 10 mL of distill water, the coupling component solution was made. The diazonium salt was added dropwise to this mixture. Thee precipitate was then filtered, washed with water, and recrystallized using absolute ethanol [18].

Compound [S1]: Molecular formula $C_{15}H_{12}O_5N_2$, mwt.: 300, color: orange-red, yield: 93% , m.p.: 168-170°C.

FT-IR (cm^{-1}), C-H $_{Arom}$ (3072), C=C $_{Arom}$ (1510), C=O$_{Ketone}$(1650), C=O $_{Carboxylic}$ (1691), and N=N(1604).

^{1}H-NMR (ppm), C-H$_{Aliph}$ (2.6), C-H$_{Arom}$ (6.8-8), and H-OH (5.6).

^{13}C-NMR (ppm), C-H$_{Alpha}$ (26.7), 12C-C=C (120- 140), C=O $_{Ketone}$ (196), and C=O $_{Carboxylic}$(162).

Synthesis of E)-(2,4-dihydroxy-5-((Z)-3-(4-hydroxy-3-methoxyphenyl) acryloyl)phenyl)diazenyl)benzoic acid[S2]

(0.01 mole , 3 g) of azo compound is dissolve in absolute ethanol and 30% NaOH in a mixture of ethanol and distilled water, was added as dropwise to azo with a stirrer. After that (0.01 mole, 1.5 g) of aldehyde (4-hydroxy-3-methoxy Benzaldehyde) was added. The mixture was stirred for 6 hours and the sediment was left in the refrigerator overnight. Thee precipitate was then filtered, washed with water, and recrystallized using absolute ethanol [19].

Compound [S2]: Molecular formula $C_{23}H_{18}O_7N_2$, mwt.: 418, color: dark-red, yield: 91%, m.p.: 149-150 °C.

Synthesis of 2,4-dihydroxy-5-(6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)diazenyl)benzoic acid [S3]

Chalcone [S2] (0.01 mol, 3 g), urea (0.01 mol, 0.6 g), and sodium hydroxide solution (10%, 5 mL) were mixed in 30 mL of pure ethanol and agitated for 4 hours. The mixture was then transferred to 20 mL of cold water with continuous stirring for 1 hour, and left overnight.

Then obtained precipitate was filtered, washed, and recrystallized using ethanol. [20].

Compuond [S3]: Molecular formula $C_{24}H_{19}O_7N_4$, mwt.: 475, color: black, yield: 83%, gooey.

The FTIR spectrum exhibited absorption band at (1508) cm^{-1} for N=N and loss absorption band at (3363, 3473) cm^{-1} for NH_2.

Synthesis of 4-((2,4-dihydroxy-5-(6-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)diazenyl)benzoic acid[S4]

Chalcone [S2] (0.01 mole, 3 g), thiourea (0.01 mole, 0.6 g), and sodium hydroxide solution (10%, 5 mL) were combined and mixed for 4 hours before being transferred to 20 mL of cold water and swirled continuously for 1 hour. The combination was then left overnight.

The precipitate was next filtered, water rinsed off it, and absolute ethanol was used to recrystallize it [21].

Compuond [S4]: Molecular formula $C_{24}H_{19}O_6N_4S$, mwt.: 492.5, color: light-red, yield: 89%, m.p.: 49-50 °C.

The FTIR spectrum exhibited absorption band at (1508) cm^{-1} for N=N and loss absorption band at (3363, 3473) cm^{-1} for NH_2.

IR (ν, cm^{-1}): O-H (3149),C-H $_{Ar}$ (3072), $C=O_{carboxylic\ acid}$ (1690), $C=C_{Ar}$ (1516), N=N (1606), C=S(1178), N-H (3406), ^{1}H-NMR (400MHz, DMSO-d_6): (δ, ppm) spectrum showed appearance signal at 6.86 for (H,OH) and signal at 9.77 for (H,COOH).

^{13}C-NMR (400MHz, DMSO-d_6): (δ, ppm) spectrum showed signals at 110-170 for Ar-C and signal at 191 for C=O $_{carboxylic\ acid}$, and signal at 56 for (C-N).

Synthesis of 4-((2,4-dihydroxy-5-(5-(4-hydroxy-3-methoxyphenyl)-1H-pyrazolE-3-yl)phenyl)diazenyl)benzoic acid[S5]

Chalcone [S2] (0.01 mole, 3 g) was dissolved in ethanol (99%, 10 mL) and (1.0 g) NaOH and refluxed with excess of ($NH_2NH_2.H_2O$) (5-10 ml) for (12 hours) at 75 °C. After filtration, washing with water, and recrystallization with 99% ethanol, the precipitate was prepared [22].

Compuond [S5]: Molecular formula: $C_{23}H_{17}O_6N_4$, mwt.: 445, color: orange, yield:85%, m.p.: 55-57°C.

Synthesis of4-((2,4-dihydroxy-5-(5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)diazenyl)benzoic acid[S6]

To a solution of sodium hydroxide, chalcone (0.01 mole, 3 g) hydroxylamine hydrochloride (0.025 g) in 0.25 mL of water in 5 mL of 99% ethanol) was added (0.05 g of NaOH in 0.5 mL of water). After cooling to ambient temperature and being poured onto crushed ice, the liquid was refluxed in a water bath for 8 hours. The precipitate was then filtered, washed with water, and recrystallized using absolute ethanol [23].

Compound [S6]: Molecular formula: $C_{23}H_{16}O_7N_3$, mwt.: 464, color: black, yield: 88%, m.p.: 50-53 °C.

Results and discussion

The first reaction in Scheme 1 involves the reactions of *p*-amino benzoic acid as nucleophile (rich in electron) with nitrous ion to form diazonium salt, after (15 min), this diazonium reaction was done with coupling compound. The reaction should be carried out

at (0-5 °C) with continuous stirring to form azo dye. The FTIR spectrum exhibited absorption band at (1508) cm^{-1} for N=N and disappearance absorption band at (3363, 3473) cm^{-1} for NH_2. The ^{1}H-NMR show signal at 8.27 for carboxylic hydrogen, 2.6 for the aliphatic hydrogen and signal at 6.98 for hydroxyl goup. The ^{13}C-NMR shows signal at (140-120 cm^{-1}) for several carbon atoms and signal at 26.72 for aliphatic carbon.

Chalcone [S2] synthesized from azo dyes with aldehyde such as vanillin (4-hydroxy-3-methoxy benzaldehyd) in the presence of ethanol as solvent and (10% NaOH, dissolved in a mixture of ethanol and distilled water) as catalyst. When the hydroxyl group accepts the hydrogen atom in the carbonyl group's alpha position, an aldol condensation process takes place, resulting in the release of a water molecule. As can be seen in the following tables, it was found that the absorbance was shifted due to the entry of a withdrawn group, which is the ether group (C-OR), in an aldehyde to synthesis chalcon. ^{1}H-NMR shows signal at (9.7), while the signal of carboxyl group was in (8.2) and this is also the evidence of occurrence of a relative displacement of the signal for the same reason mentioned above, ^{13}C-NMR showed signals at (120-175) for Ar-C. This rate was due to the presence of more than one aromatic ring, and each ring has a different compensation, which made the displacement in close places, signal at (191) for C=O $_{\text{carboxylic acid}}$ and signal at (26.72) for C-H $_{\text{alpha}}$.

TABLE 1 FT-IR spectral data (cm^{-1}) of compounds (S1, S2)

No. of comound	*ν*(C-H) Arom	*ν*(C=C) Arom	*ν*(C=O) Keton	*ν*(C=O) Carboxylic	*ν*(N=N
S1	3072	1510	1650	1691	1604
S2	3040	1550	1683	1699	1604

TABLE 2 ^{1}H-NMR spectral data (ppm) of compounds (S1, S2)

No. of compound	(C-H$_{Arom}$)	(O-H)	C-H$_{Alpha}$
S1	6 – 8	5.6	3.6
S2	6.9-8	6.8	3.4

TABLE 3 ^{13}C-NMR spectral data (ppm) of compounds (S1, S2)

No of compound	(C=C$_{Arom}$)	(C-OH)	C-H$_{Alpha}$	C=O$_{Carboxylic}$	C=O$_{Ketone}$
S1	120-140	154.5	26.72	196.5	167.5
S2	120-175	154.0	26.72	191	196

(Oxazine S3 and Thiazen S4) were prepared from the reaction of (urea and thiourea) with ethanol in the presence of a few drops of sodium hydroxide with continuous stirring in an ice bath, and heterocyclic compounds with a hexagonal ring were produced when the chalcone interacted with urea as a result of that a viscous compound, while a powdery compound was produced when it interacted with thiourea. After the amine group disappeared in the first reaction, it reappeared in compounds [S3,S4] (3331, 3423) as a result of the interaction of chalcone with urea and thiourea, and the IR technique showed this through the values recorded in Table 4. ^{1}H-NMR spectrum showed appearance signal at (8.05, 8.12) for (H,NH$_{Hetero}$) and signal at 9.77 for (H,COOH) . ^{13}C-NMR spectrum showed signals at (110-160-170) for Ar-C and signal at 191 for C=O carboxylic acid, disappearance signal at 26.72 for C=C $_{\text{ailpha}}$, and appearance signal at (56) for (C-N).

TABLE 4 FT-IR spectral data (cm^{-1}) of compounds (S3, S4)

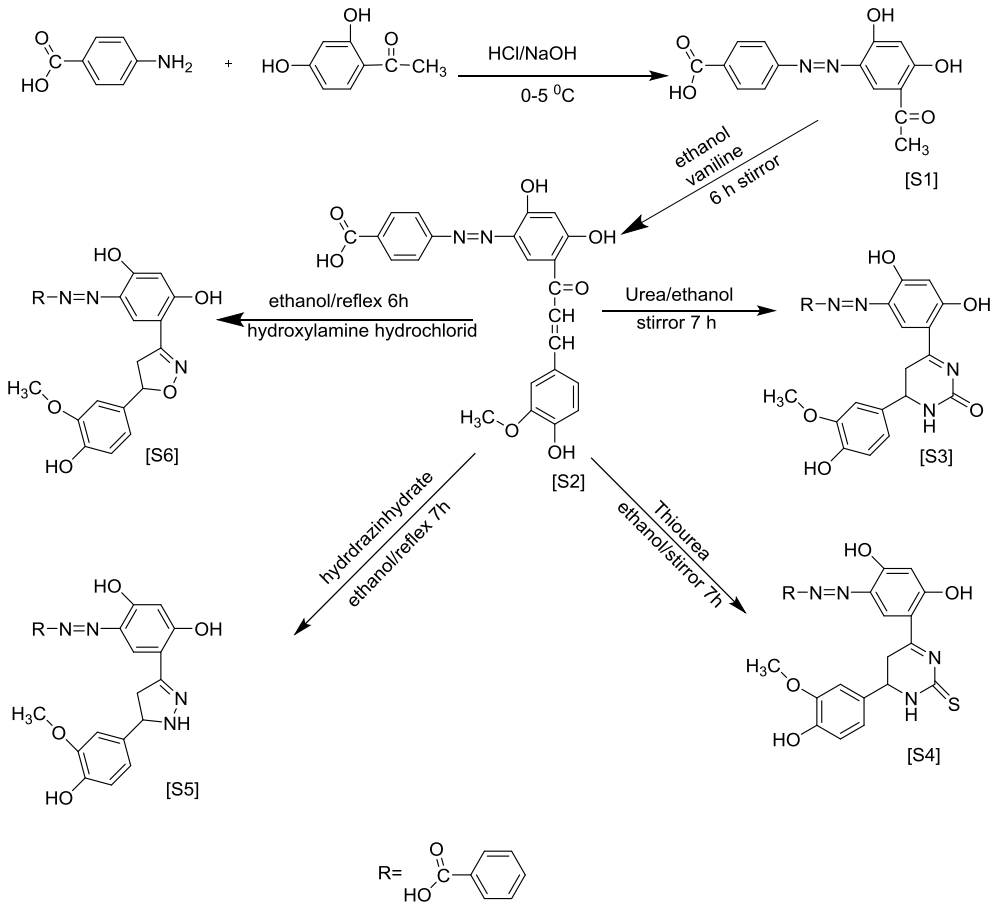
No. of compound	ν(C-H) Arom	ν(C=C)Arom	ν(N-H)	ν(C=O)Carboxylic	ν(N=N
S3	3030	1541	3331	1695	1595
S4	3040	1516	3423	1690	1606

(Pyrazol S5 and Isoxazol S6) compounds with a five-ring were prepared from the interaction of (hydrazine hydrate and hydroxylamine hydrochloride) compounds with ethanol in the presence of a few drops of sodium hydroxide with the presence of a reflex reaction. Paraffin oil was used during the reflex process so that the heat is distributed evenly on all parts of the round flask. These two reactions formed five heterogeneous cyclic compounds. Through the experiment, it turned out that the compound isoxazole [S6] is a somewhat viscous compound, so there was difficulty in dealing with it. The data about FT-IR spectrum is shown in Table 5, ^{1}H-NMR Spectrum showed appearance muti signal at (7.8) for hetero and aromatic ring and signal at (6.17) for (H,OH) and signal at (9.24, 8.5) for (H).

^{13}C-NMR spectrum showed signals at (110-160) for Ar-C, signal at 186 for C=O $_{\text{carboxylic acid}}$ and disappearance signal at 26 for C-H $_{\text{alepha}}$, and appearance signal at (56,55) for C-N.

TABLE 5 FT-IR spectral data (cm^{-1}) of compounds (S5, S6)

No. of compound	ν(C-H)Arom.	ν(C=C)Arom	ν(N-H)	ν(C=O)Carboxylic	ν(N=N
S5	3032	1545	3416	1650	1604
S6	3030	1541	-	1654	1581



SCHEME 1 Synthesis of compounds [S1-S6]

Biological activity

In this study, the activity of some types of bacteria *Staphylococcus epiderimidis, Pseudomonas* (gram-positive), *Klipsiella,* and *E.coli* (gram-negative) were measured by an agar disc, where the compounds dissolved in dimethyl sulfoxide (DMSO) at a concentration of (10^{-3} M). It was observed that (Figure 2, Table 6) the compounds differed in their sensitivity according to the different types of bacteria. In the gram-positive (*pseudomonas)* bacteria, compound [S4] appeared to be more effective than the rest of the compounds because it contained sulfur in its composition, while this effect was less with Italic. While in the negative gram, the compound [S5] showed a little effect on (*E.coli)* bacteria, while the same compound showed a high effect on (*Klipsiella*) bacteria due to the presence of the nitrogen element in a greater amount. According to the results the compounds that had a strong anti-bacterial action because of their structural contact with certain bacteria's cell walls resulted in high inhibition.

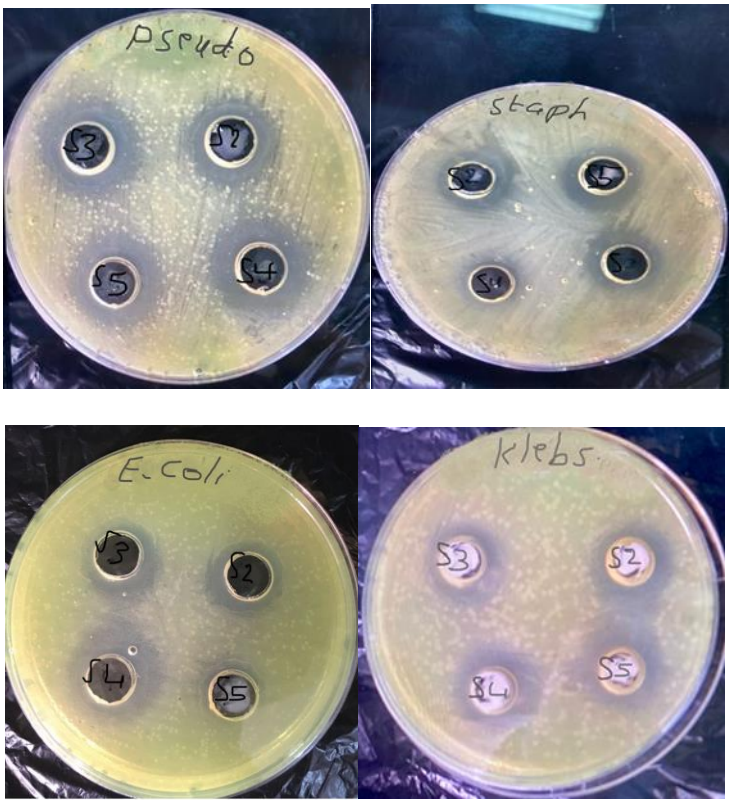


FIGURE 2 Effect of compounds [S2-S5] on *Staphylococcus Epiderimidis*, *pseudomonas*, *Klebsiella pneumoniae, and E.Coli.*

TABLE 6 Antibacterial activity of the compounds [S2-S5]

No. of compound	*Staphylococcus Epiderimidis*	*pseudomonas*	*Klebsiella pneumoniae*	*E.Coli*
S2	27	22	25	23
S3	26	22	19	23
S4	9	33	26	30
S5	25	23	25	11

Conclusion

In this experiment, azo dye was synthesized from diazonium salts with aromatic phenol undergo an azo coupling reaction that yields dark-colored compounds (azo dye). Azo dye as ketone and aldehyde (vanillin) inters aldol condensation reaction to produce chalcone derivatives.

Chalcone react with deferent compounds (urea, thiourea, hydrazine hydrate, and hydroxyl amine hydrochloride) to produce (oxazine, thiazen, pyrazol, and isoxazol) derivatives, respectively.

The prepared compounds showed biological activity against some types of bacteria, such as (*Staphylococcus Epiderimidis*, *Pseudomonas*, *Klebsiella pneumoniae, and E.Coli*). By studying the effectiveness of the prepared compounds, it was found that compound [S4] was more effective on bacteria than its peers because it contains sulfur in its chemical composition, which clearly affected the cell walls of these bacteria and showed a high zoom.

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Conflict of Interest

The authors declare no conflict of interest.

Orcid:

Sura Sadiq Obaid

https://orcid.org/0000-0003-0711-3211

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