IJMCM 12(2) (2022) 1713-1721 Doi: 10.22034/IJMCM.2022.694299

International Journal of
Molecular and Clinical Microbiology

Research Article

Synthesis and evaluation of the antibacterial activity of benzo [5,6] chromeno[3,2-c] quinoline derivatives

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ARTICLE INFO

Article history:
Received 14 March 2022
Accepted 23 August 2022
Available online 1 September 2022

Keywords:
Antibacterial activity;
Chromene;
Minimum inhibitory concentration (MIC)

ABSTRACT

The emergence and spread of antibiotic resistance in pathogenic bacteria have become a major challenge for human health, necessitating the need to synthesize new compounds against these bacteria. Chromens are among the compounds that are candidates for the production of antimicrobials due to their therapeutic properties. Ten novel 4-H pyran derivatives (4a-j) were synthesized via One-Pot, the three-components reaction of various arylglyoxal monohydrates, 2-naphthole, and quinoline-2, 4 dione in H_2O: EtOH (2:1), as a green solvent, in the presence of p-Toluene sulfunic acid, as a mild catalyst, under reflux conditions with considerable yields. The structure of new compounds was elucidated using 1H-NMR, 13 C-NMR, FT-IR, mass spectral data and HRMS. The newly synthesized compounds were evaluated for them in-vitro antibacterial activity against some pathogenic bacteria, using the agar well diffusion method. Minimum inhibitory concentration (MIC) values were determined using the microplate method. Compounds g, h, i and j show more efficiency than Ampicillin (as a reference antibiotic) while a, b, c, d, e, and f show slight activity against tested bacteria. Increasing concentration leads to more antimicrobial activity. The studied compounds had a higher antibacterial effect against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) than gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* bacteria. Some of the synthesized compounds in this study showed good antibacterial potential against the studied microorganisms, which can be used against these bacteria if confirmed in In-vivo experiments.

1. Introduction

Poly-functionalized 4-H pyran nucleus is a prolific source of important molecules with a wide range of interesting biological and pharmaceutical activities. Chromene structures had an effective role in pharmaceutical research in recent years and they have been used as precursors in synthesizing pharmaceutically active compounds (Shehab *et al*., 2011). Based on the scientific references and databases, recent researches have focused on the recognition and development of diverse and useful applications of these beneficial compounds such as antimicrobial (Sari et al., 2017; Keiko *et al.*, 2002) , anti-viral (Mohammadi *et al.*, 2013; Gurdal *et al.,*2009), antioxidant (Aytemir et al., 2010; Shanti et al., 2009), anti-diabetic (Soni *et al*., 2016), anti-allergic, anti-rheumatism, anti-spasm (Domarle et al.,1997), anti-cancer (Wang

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et al., 2016; Jain *et al*., 2019), anti-HIV protease (Douglas et al.,1999), anti-convulsion (Aytemir et al., 2004), anti-schizophrenia (Konkoy et al., 2001), psychotropic, anti-inflammatory (Magesh *et al*., 2004), cardiovascular stimulant (Liu et al., 1996), anti-Malaria and protective effects against neurological disorders including Alzheimer's and Parkinson. Therefore, the synthesis of such compounds has attracted a lot of interest nowadays.

Antimicrobial properties of chromene molecules have been studied by many scientists such as Khafagy *et al*. 2002, Damljanovic *et al.* 2009, Costa *et al*. 2016, and Youssef *et al.* 2017. Also, antiviral activities of 4-H pyran derivatives have been studied by various researchers. (Smith *et al.*, 1998; Hashem *et al*., 2007; Farghaly *et al.*, 2006).

During the past five decades the use and misuse of antimicrobials in both humans and veterinary has resulted in the emergence of strains of bacteria that no longer respond to antimicrobial therapy. Antimicrobial resistance is one of the most urgent priorties in the fields of public health today. Due to the resistance of pathogenic bacteria against the most common antibiotics, there is an urgent need to identify new chemicals with antimicrobial properties.

The aim of this study was to investigate the antibacterial activities of newly synthesized chromene derivatives against gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic bacteria.

2. Materials and Methods

2.1. Synthesis and characterization of benzo [5,6] chromeno [3,2-c] quinoline derivatives

A new series of benzo [5,6] chromeno[3,2-c] quinoline derivatives were successfully synthesized using a convenient One-Pot multicomponent reaction method based on green synthesis. It was done using various arylglyoxal monohydrates (1a-j), quinoline-2,4-dione (3), and *ß*-naphthol (2) in H_20:EtOH (2:1) as a green solvent in the presence of *p*-toluene sulfonic acid as a mild catalyst under reflux conditions with high yields (83-92%). The reaction conditions were optimized using different solvents at variable thermal conditions. The optimized conditions for this synthesis have been reported in our previous study (Sadeghpourorang et al. 2021). The target (4a-j) compound was synthesized based on the reaction shown in Figure 1. Structures of all new products were defined by H-NMR, BC-NMR, FT-IR, mass spectral data, and HRMS.

The chemicals and reagents used for the synthesis were obtained from Merck and Sigma Aldrich companies. Melting points were measured using an Electrothermal 9,200 apparatus. Infrared spectra were measured by spectrum Tensor 27, Bruker, and Equinox 55 FT-IR instrument using KBr disks. IH (500 MHz) and 13C (125 MHz) NMR spectra were recorded using a Varian-Inova spectrometer in DMSO-d6 with TMS as an internal reference. Analytical thin-layer chromatography (TLC) was carried out on a pre-coated aluminum sheet with silica gel 60 F 254 (Merck, Germany) and the detection was made by a UV lamp (1254 nm). Mass analysis was performed using an Agilent Technology (HP) 5,973 Network Mass Selective Detector, and high-resolution mass spectra were recorded on a Kratos mass spectrometry (MS) 25RF spectrometer. The structure of newly synthesized compounds is shown in Table 1.

2.2. Evaluation of antimicrobial activity

From the synthesized compounds, different concentrations of 1.56 ,3.125 ,6.25, 12.5, 25, 50, 100 mg/ml were prepared using 5% DMSO (Dimethyl sulfoxide) for use in the well diffusion test and determining the minimum inhibitory concentration and the minimum bactericidal concentration (MIC/MBC). Solutions were filtered with a millipore size of 0.2 µm.

2.3. Evaluation of antimicrobial activity

From the synthesized compounds, different concentrations of 1.56, 3.125 ,6.25, 12.5, 25, 50, 100 mg/ml were prepared using 5% DMSO (Dimethyl sulfoxide) for use in the well diffusion test and determining the minimum inhibitory concentration and the minimum bactericidal concentration (MIC/MBC). Solutions were filtered with a millipore size of 0.2 µm.

2.4. Preparation of bacterial strains

Lyophilized standard bacterial strains were obtained from the microbial collection of Iranian Research organization for science and Technology (IROST). Microbial samples were regenerated according to standard methods. To prepare a microbial suspension from the fresh and young bacterial culture, it was transferred to Müller-Hinton Broth and incubated for 2 hours at 37°C to obtain turbidity similar to 0.5 McFarland (1.5×10^8 cfu/ml).

The bacterial strains used in this study included *Staphylococcus aureus* (ATCC: 25923), *Bacillus cereus* (PTCC: 1052), *Escherichia coli* (ATCC: 25922) and *Pseudomonas aeruginosa* (ATCC: 27853).

2.5. Determination of antibacterial activity

Agar well diffusion method was used for this purpose. Firstly, a sterile cotton swab was inserted into the bacterial suspension (with turbidity equivalent to 0.5 McFarland) and the additional amount of suspension was taken by pulling the swap to the tube wall, and the swab was streaked uniformly on Müller-Hinton agar plates. Then, wells with a diameter of 5 mm and a distance of 2 cm were created on the surface of plate. Each well was filled with 100, 50, 25, 12.5 mg/ml dilutions of the synthesized compounds.

Ampicillin was used as a positive control and DMSO was used as a negative control. All culture media were incubated for 24 hours at 37°C. After this period, bacterial cultures were examined for the formation of a growth inhibition zone around the well (in millimeters). If there was a growth inhibition zone, it was measured by caliper.

The diameter of the growth inhibition zone is a reaction to the concentration of the test compound. This phenomenon is a linear relationship between the inhibition zone and the logarithm of the concentration of the test compound, which can be done by measuring the diameter of zone and comparing it with a specific standard (CLSI 2020).

2.6. Determination of MIC/MBC

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that can inhibit the visible growth of a microorganism. Determination of minimum inhibitory concentrations is an important method in diagnostic laboratories to confirm the resistance of microorganisms to an antimicrobial agent and monitor the activity of new antimicrobial agents (CLSI 2020).

The Micro titer plate method using Resazurin reagent was used to determine the minimum inhibitory concentration (MIC). Standard microplate contains 96 wells, each containing a volume of 0.1 mL were used for this test. For this purpose, first, 100 microliters of Mueller Hinton broth were poured into all the wells. Then, two-fold serial dilutions of the tested substances and the control antibiotic were made in the wells. Then, a dilution equal to one hundredth of 0.5 McFarland's was prepared from the studied bacteria and added to all wells. Finally, 10 microliters of resazurin reagent was added to all wells and the microplates were incubated at 37°C for 24 hours (Elshikh *et al*., 2016).

To determine the minimum bactericidal concentration (MBC), the studied compounds were collected from the sample as the minimum inhibitory concentration and two previous samples were cultured in Müller-Hinton agar medium. The minimum concentration with no visible bacterial growth was recorded as MBC.

Each experiment was performed in triplicate for each bacterium and the results were analyzed by SPSS software version 21 using Student T-test at a significant level of $p \leq 0.05$.

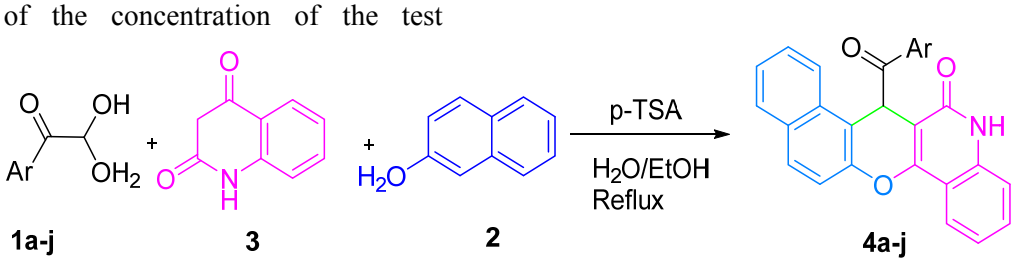


Figure 1. Synthesis of benzo [5,6] chromeno[3,2-c] quinoline derivatives

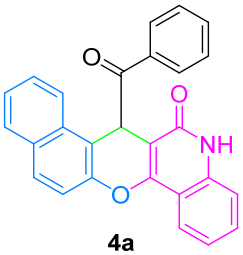
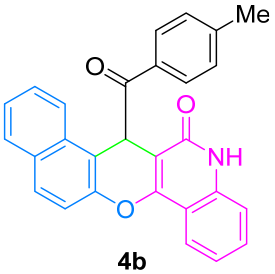
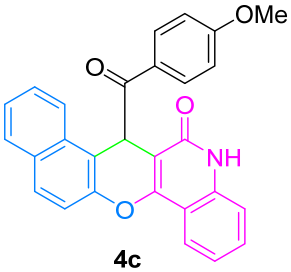
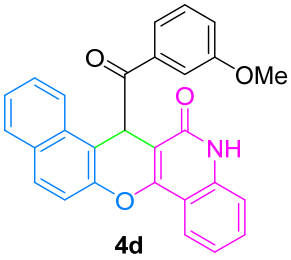
3. Results

To optimize the reaction conditions, this study was started with the synthesis of benzo [5,6] chromeno [3,2-c] quinoline derivatives by a systematic study on the model reaction of arylglyoxals, quinolone-2,4(1H,3H) – dione, and naphthalene-2-ol (molar ratio 1:1:1) using various solvents, catalysts, times, and temperatures to evaluate the rate and the yield of reactions. The results are mentioned in our previous study (Sadeghpour *et al.*, 2021).

Some of the studied synthetic compounds had significant effects on the used bacteria in comparison with the control antibiotics.

The greatest effect was observed against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* with the combination of 10 (6-hydroxy-7H-benzo [5,6] cromeno [3,2-c] quinoline-7-IL) (4). Nitrophenyl (methanone) and after compounds 7 and 8, respectively. With increasing the concentration of the compound, its inhibitory effect also increased. Compounds 1 and 2 did not show a slight inhibitory effect on any of the studied bacteria. Compounds 5, 3 and 4 at a concentration of 100 mg/ml showed much less antibacterial activity against the studied gram-positive bacteria than control antibiotic. (The results are listed in Table 2).

Table 1. The structure of compounds (4a-j)

Entry	Products	Name	^{1}HNMR Data
1 (a)	 **4a**	7-benzoyl-5,7-dihydro-6H benzo[5,6]chromeno[3,2-c]quinolin-6-one	White powder; mp: 299-300 °C^{1}H NMR (500 MHz, DMSO-d_6) δ 12.56 (s, 1H, OH, D_2O exch.), 12.17 (s, 1H, OH, D_2O exch.), 7.98 (d, *J* = 7.7 Hz, 2H, Ar), 7.70 (d, *J* = 7.4 Hz, 2H, Ar), 7.59 (t, *J* = 7.3 Hz, 2H, Ar), 7.44 (t, *J* = 7.3 Hz, 2H, Ar), 7.39–7.34 (m, 3H, Ar), 7.33-7.28 (m, 3H, Ar), 6.35 (s, 1H, Ar) 1.
2 (b)	 **4b**	(E)-7-(hydroxy(p-tolyl)methylene)-7H-benzo[5,6]chromeno[3,2-c]quinolin-6-ol	White powder; mp: 297-298 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 12.53 (s, 1H, OH, D2O exch.), 12.16 (s, 1H, OH, D2O exch.), 7.98 (d, J = 8.1 Hz, 2H, Ar), 7.59 (t, J = 8.3 Hz, 4H, Ar), 7.37 (d, J = 8.2 Hz, 2H, Ar), 7.30 (t, J = 7.5 Hz, 3H, Ar), 7.14 (d, J = 7.9 Hz, 2H, Ar), 6.31 (s, 1H, Ar), 2.25 (s, 3H, Me).
3 (c)	 **4c**	7-(4-methoxybenzoyl)-5,7-dihydro-6H-benzo[5,6]chromeno[3,2-c]quinolin-6-one	White powder; mp: 281-282 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 12.51 (s, 1H, OH, D2O exch.), 12.15 (s, 1H, OH, D2O exch.), 7.98 (d, J = 8.0 Hz, 2H, Ar), 7.68 (d, J = 8.6 Hz, 2H, Ar), 7.59 (t, J = 7.4 Hz, 2H, Ar), 7.38 (d, J = 8.0 Hz, 2H, Ar), 7.30 (t, J = 7.4 Hz, 3H, Ar), 6.89 (d, J = 8.7 Hz, 2H, Ar), 6.28 (s, 1H, Ar), 3.74 (s, 3H, OMe).
4 (d)	 **4d**	(E)-7-(hydroxy(3-methoxyphenyl) methylene)-7H-benzo[5,6]chromeno[3,2-c]quinolin-6-ol	White powder; mp: 294 °C (Dec.); ^{1}H NMR (500 MHz, DMSO-d6) δ 12.52 (s, 1H, OH, D2O exch.), 12.16 (s, 1H, OH, D2O exch.), 7.96 (d, J = 8.2 Hz, 2H, Ar), 7.57 (t, J = 7.5 Hz, 2H, Ar), 7.35 (d, J = 8.1 Hz, 2H, Ar), 7.28 (t, J = 9.5 Hz, 2H, Ar), 7.23 (m, 4H, Ar), 7.00 (bs, 1H, Ar), 6.32 (s, 1H, Ar), 3.64 (s, 3H, OMe).

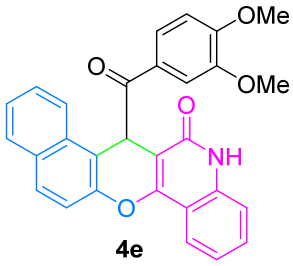
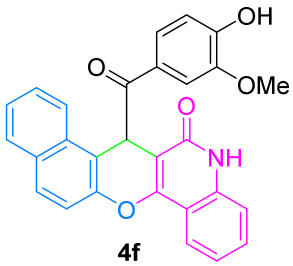
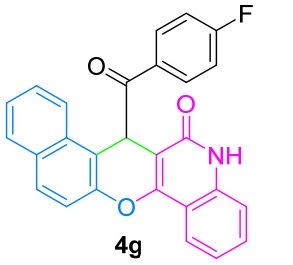
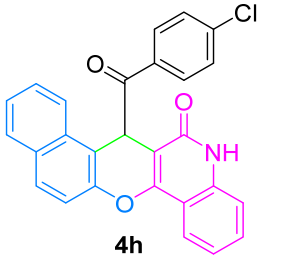
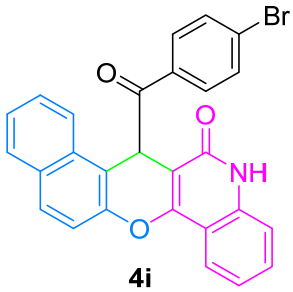
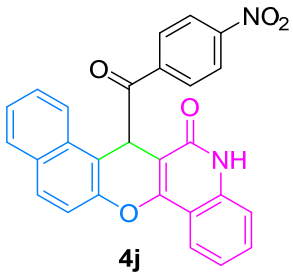
5 (e)	 **4e**	(E)-7-((3,4-dimethoxy phenyl)(hydroxy)methylene)-7H-benzo[5,6] chromeno[3,2-c]quinolin-6-ol	White powder; mp: 274-275; ^{1}H NMR (500 MHz, DMSO-d6) δ 11.72 (s, 1H, OH, D2O exch.), 10.59 (s, 1H, OH, D2O exch.), 8.04 (d, J = 7.6 Hz, 1H, Ar), 7.96 (d, J = 7.8 Hz, 1H, Ar), 7.93 – 7.85 (m, 3H, Ar), 7.64 (s, 1H, Ar), 7.42 (d, J = 33.3 Hz, 3H, Ar), 7.33 – 7.24 (m, 3H, Ar), 7.03 (d, J = 8.4 Hz, 1H, Ar), 3.76 (s, 3H, OMe), 3.58 (s, 3H, OMe).
6 (f)	 **4f**	(E)-7-(hydroxy(4-hydroxy-3-methoxyphenyl) methylene)-7H-benzo[5,6]chromeno[3,2 c]quinolin-6-ol	White powder; mp: 288-289 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 12.54 (s, 1H, OH, D2O exch.), 12.16 (s, 1H, OH, D2O exch.), 9.80 (s, 1H, OH, D2O exch.), 7.98 (d, J = 7.8 Hz, 2H, Ar), 7.59 (t, J = 7.7 Hz, 2H, Ar), 7.38 (d, J = 8.1 Hz, 2H, Ar), 7.33-7.27 (m, 4H, Ar), 7.20 (d, J = 8.0 Hz, 1H, Ar), 6.68 (d, J = 8.3 Hz, 1H, Ar), 6.26 (s, 1H, Ar), 3.60 (s, 3H, OMe).
7 (g)	 **4g**	(E)-7-((4-fluorophenyl)(hydroxy)methylene)-7H-benzo[5,6]chromeno[3,2-c]quinolin-6-ol	White powder; mp: 294 °C (Dec.) °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 12.53 (s, 1H, OH, D2O exch.), 12.18 (s, 1H, OH, D2O exch.), 7.98 (d, J = 8.1 Hz, 2H, Ar), 7.78-7.75 (m, 2H, Ar), 7.59 (t, J = 7.7 Hz, 2H, Ar), 7.37 (d, J = 8.2 Hz, 2H, Ar), 7.30 (t, J = 7.7 Hz, 3H, Ar), 7.19 (t, J = 8.6 Hz, 2H, Ar), 6.36 (s, 1H, Ar).
8 (h)	 **4h**	7-(4-chlorobenzoyl)-5,7-dihydro-6H-benzo[5,6]chromeno[3,2-c]quinolin-6-one	Light yellow powder; mp: 211-212 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 11.71 (s, 1H, OH, D2O exch.), 8.94 (d, J = 8.3 Hz, 1H, Ar), 7.97 (d, J = 8.7 Hz, 1H, Ar), 7.93 (d, J = 8.2 Hz, 1H, Ar), 7.80 (d, J = 8.0 Hz, 1H, Ar), 7.61 (t, J = 8.4 Hz, 3H, Ar), 7.56 (t, J = 8.7 Hz, 3H, Ar), 7.46 (d, J = 8.8 Hz, 1H, Ar), 7.41 (t, J = 8.5 Hz, 2H, Ar), 7.25 (t, J = 7.6 Hz, 1H, Ar), 5.54 (s, 1H, CH).
9 (i)	 **4i**	(E)-7-((4-bromophenyl)(hydroxy)methylene)-7H-benzo[5,6] chromeno[3,2-c]quinolin-6-ol	White powder; mp: 293 °C (Dec.);; ^{1}H NMR (500 MHz, DMSO-d6) δ 12.52 (s, 1H, OH, D2O exch.), 12.18 (s, 1H, OH, D2O exch.), 7.97 (d, J = 7.9 Hz, 2H, Ar), 7.64–7.55 (m, 7H, Ar), 7.37 (d, J = 8.2 Hz, 2H, Ar), 7.30 (t, J = 7.6 Hz, 2H, Ar), 6.36 (s, 1H, Ar).
10 (j)	 **4j**	(6-hydroxy-7H-benzo [5,6]chromeno[3,2-c]quinolin-7-yl)(4-nitrophenyl)methanone	Light yellow powder; mp: 224-225 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ 11.72 (s, 1H, OH, D2O exch.), 8.93 (d, J = 8.4 Hz, 1H, Ar), 8.30 (d, J = 8.7 Hz, 2H, Ar), 7.97 (d, J = 8.9 Hz, 1H, Ar), 7.92 (d, J = 8.3 Hz, 1H, Ar), 7.86 (d, J = 8.7 Hz, 2H, Ar), 7.79 (d, J = 7.8 Hz, 1H, Ar), 7.61–7.52 (m, 2H, Ar), 7.46 (d, J = 8.9 Hz, 1H, Ar), 7.40 (t, J = 7.7 Hz, 2H, Ar), 7.24 (t, J = 7.4 Hz, 1H, Ar), 5.62 (s, 1H, CH).

Table 2. Minimum inhibitory concentration (MIC) values of the synthesized compounds on the studied bacterial strains

Pseudomonas aeroginisa	*Escherichia coli*	*Bacillus cereus*	*Staphylococcus aureus*	**compounds**
12.5	6.25	3.12	0.39	Ampicillin
50	12.50	6.25	0.78	a
25	25	12.5	0.78	b
50	12.5	6.25	0.39	c
12.5	6.25	3.12	1.56	d
12.5	12.5	3.12	3.12	e
6.25	12.5	6.25	0.78	f
12.5	6.25	3.12	0.39	g
6.25	6.25	1.56	0.39	h
25	6.25	3.12	0.39	i
-	-	12.5	12.5	j

4. Discussion

The increase of antibiotic-resistant microorganisms, especially multidrug-resistant bacteria, requires the discovery and use of new compounds that can act against these bacteria.

Chromen based derivatives, due to their various chemical and biological properties, are highly regarded and are among the compounds whose various therapeutic properties have been proven and can help humans in this way (Montazeri *et al*., 2017).

In this study, 10 compounds with the structure of benzo [5,6] chromeno [3,2-c] quinoline were synthesized by the 3-component monolayer method.

The selected method was one of the best available methods for the synthesis of biologically active heterocycles in recent years due to several advantages such as using green strategy and solvent, avoiding unwanted and undesirable by-products and having environmentally sustainable conditions. This not only prevents unnecessary syntheses but also limits the number of derivatives with biologically-compelling behaviors.

Evaluation of the antibacterial properties of newly synthesized compounds using Agar well diffusion method showed that some of these compounds have effective antibacterial activities against the studied bacteria, especially gram-positive bacteria.

In a study performed by Montazeri on the synthesis of 3, 4 dihydropyran and c-chromen derivatives, it was shown that two of the synthesized compounds had acceptable antibacterial activity against *Escherichia coli* compared to Penicillin. Other synthesized compounds also showed an acceptable effect on all studied strains (Montazeri et al., 2017).

Sangani *et al*. synthesized Pirano [2,3-c] chromen derivatives from pyrazole compounds and it was found that some of these synthesized substances are even stronger than commercial antibiotics (Sangani et al., 2011).

In another study performed by Al-Saghir *et al*. (34), was shown that new Pirano [4,3-c] and Cromeno benzo [c] chromen derivatives had antimicrobial effects from medium to high compared with the initial substrate against *Salmonella* Typhi, *Bacillus subtilis, Staphylococcus aureus* (Al-Saghir *et al*., 2007).

In another study, the synthesized chromen compounds showed significant antibacterial activity against gram-positive bacteria, which is similar to the effects observed in the present study (Mirnejad *et al.,* 2013*)*.

In a previous study on synthesized pyran compounds and the combination of furan and pyran derivatives, significant effects were observed in comparison with reference antibiotics on the studied bacteria, including *Staphylococcus aureus* (Shehab, 2011).

The differences observed in previous studies can be due to differences in the functional groups, the method of synthesis of compounds and the type of bacterial strains studied.

In the present study, the synthesized compounds showed stronger antibacterial effects against gram-positive bacteria compared to gram-negative bacteria, which may be due to differences in the cell wall structure of these bacteria.

The difference in the levels of the antibacterial activity of synthesized compounds can be related to different functional groups in

synthesized compounds, that compounds with nitro and halogen functional groups showed a higher antibacterial effect.

Conclusion

The results of the present study showed that some of the newly synthesized compounds have antibacterial activity against the studied bacteria. These effects are promising to find new compounds to fight microbes, especially resistant strains to antimicrobial drugs if approved in additional experiments.

Acknowledgment

The authors gratefully acknowledge for the support of the research officials of Islamic Azad University Ahar Branch

DECLARATIONS

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethics approvals and consent to participate

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

Refereces

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