Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.57122

Bioactivity evaluation of synthesized flavone analogs

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Abstract

Flavonoids are natural phenolic compounds found in dietary sources such as plants, fruits, and vegetables. They have a wide range of biological activities including cytostatic and anti-inflammatory effects. In this research, we synthesized flavone derivatives via the introduction of various aryl functional groups. Furthermore, we combined flavones with gallic acid derivatives. The synthesized compounds were tested against cervical (HeLa) and colon cancer cells (CaCo-2). Moreover, the synthesized compounds were evaluated for their antioxidant and anti-inflammatory activities using DPPH and COX inhibition tests, respectively. The success of the synthesis of our target compounds was confirmed using IR and NMR. Compound 3 potently inhibited the proliferation of CaCo-2 cells (IC_{50} = 2.42 μg/mL). Meanwhile, compound 4 exhibited antioxidant activity (IC_{50} = 3.53 ± 0.1 μg/mL). Moreover, compound 4 selectively inhibited COX-2 with an IC_{50} = 6.02 ± 0.33 μg/mL; it was approximately 6-fold more selective for COX-2 than for COX-1 enzymes. In conclusion, the results strongly indicate that the chemical modification of flavones enhances their bioactivity. Further investigation of in vivo anti-inflammatory and anticancer evaluation of active compounds is required to prove both safety and efficacy.

Keywords: flavone; bioactivity; anticancer.

Practical Application: This work is of interest to the pharmaceutical and food industry. Natural products such as flavones and gallic acids can be used as a semisynthetic approach for effective drugs to treat cancer and inflammation.

1 Introduction

Flavonoids are phytochemical compounds. They are natural phenol compounds that belong to the family of polyphenols found in various plants such as fruits, vegetables, and plant-derived beverages such as green tea and wine. Cocoa-based products are the main dietary source of flavonoids (Mena et al., 2016; Tungmunnithum et al., 2018). They are composed of two phenolic rings linked by an oxygen-containing heterocycle (Vaya & Tamir, 2004). Flavonoids are divided into six subclasses depending on their chemical structure. However, these six subclasses share the same two phenolic rings and the oxygen-containing heterocycle (Woodman et al., 2005). The structural variation is responsible for their wide biological activity, such as antibacterial, antiviral, analgesic, antiallergic, hepatoprotective, cytostatic, apoptotic, estrogenic, and anti-estrogenic functions (Verma & Pratap, 2010; Busch et al., 2015). Flavones have shown very noticeable antimicrobial activity (Cushnie & Lamb, 2011; Xie et al., 2015). Besides the antimicrobial activity, flavones have shown an effective anticancer activity (Raffa et al., 2017). Under normal conditions, flavones act as antioxidants and are potent pro-oxidants that trigger the apoptotic pathway and downregulate pro-inflammatory signaling pathways in cancer cells (Van Acker et al., 1996). The anticancer activity is attributed to modulating the activity of reactive oxygen species (ROS)-scavenging enzymes, arresting the cancer cell cycle, inducing apoptosis and autophagy, and suppressing cancer cell proliferation and invasiveness (Kopustinskiene et al., 2020). Moreover, flavones have demonstrated anti-inflammatory effect by inhibiting the regulatory enzymes or transcription factors that are important in controlling inflammatory mediators (Maleki et al., 2019). Flavones can modulate the neuroinflammatory response involved in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis (Spagnuolo et al., 2018).

Gallic acid is a polyphenol natural chemical present in many medicinal plant species, such as the peel of the pomegranate. It has anti-inflammatory and antibacterial activities (Li et al., 2017). Gallic acid was effective as a skeletal muscle relaxant in experimental animal models (Asdaq et al., 2021). The anti-inflammatory activity of gallic acid was evaluated in human gestational tissues. The results showed a reduction in pro-inflammatory cytokines and chemokines, as well as degradation of the extracellular matrix and matrix-remodeling enzymes (Nguyen-Ngo et al., 2020).

The importance of gallic acid and its derivatives is attributed to its antitumor activity. Methyl, lauryl, and propyl gallate induce apoptosis in tumor cell lines and inhibit lymphocyte proliferation. It has been suggested that the apoptosis induced

Received 23 June, 2022
Accepted 20 Sept., 2022
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by gallic acid and its analogs is associated with alterations in oxidative metabolism (Locatelli et al., 2009).

Chemical modification of natural compounds is a common research procedure aimed to improve the pharmaceutical, physicochemical, and biological activity of the studied compounds (Wold, 1981; Sanchez et al., 1988; Polyakov, 1999; Masina et al., 2017). In this study, we synthesized a small library of flavone analogs by the addition of an extra phenyl or benzyl aromatic group to increase biological activity. Moreover, we combined gallic acid derivatives with flavones to achieve a synergistic effect that could lead to a more potent biological effect. The biological activities of the synthesized compounds were evaluated using standard procedures.

2 Methodology

2.1 Reagents and materials

All reagents were commercially obtained and used without further purification. Boron trifluoride diethyl etherate (98% purity; catalog # A15275) was purchased from Alfa Aesar, UK. Benzylmagnesium chloride solution (catalog # 302759) and phenyl magnesium chloride solution (catalog # 224448) were purchased from Sigma-Aldrich. Acetone, methanol, dichloromethane, hexane, and ethyl acetate were purchased from C.S. Company, Haifa, Palestine. Diethyl ether (catalog # 38132) was purchased from Merck Millipore, and tetrahydrofuran (THF) (catalog # 487308) was purchased from Carlo Erba Company, MI. Italy. Sodium sulfate, ammonium chloride, and sodium bicarbonate were purchased from C.S. Company, Haifa, Palestine. COX (Human) inhibitor screening assay kit (Item # 701230) was purchased from Cayman chemicals. RPMI 1640 culture medium, trypsin, glutamine, fetal calf serum, and other reagents were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (catalog # 224448) was purchased from Sigma Aldrich, Germany.

2.2 Instrumentation

Silica gel (Merck, 230-400 mesh) was used for flash chromatography. Chromatography columns were eluted with positive air pressure. For evaporation of the solvents, Rota Vapor (Heidolph) was used. NMR analysis was conducted using Bruker Avance 500 spectrometer at Jordan University. UV-Visible spectrophotometer (JENWAY, UK) was used for anti-inflammatory test. Chemical shifts were reported in ppm, and coupling constants were reported in Hz. Accumax variable micropipette, UK, was used for pipetting. Unilab microplate reader 6000 was used for the measurement of absorbance.

2.3 Chemical synthesis and characterization of the products

Synthesis of tertiary alcohol analogs of flavones

The Grignard reagent (1.1 mmole) was added to the required flavone (1 mmole), and they were mixed in a round bottom flask under a nitrogen atmosphere at 0 °C. The mixture was stirred for 24 h and then quenched with saturated ammonium chloride (10 mL). The mixture was then extracted with ethyl acetate (3 × 20 mL), dried over sodium sulfate, and evaporated under reduced pressure. The residue was purified using silica gel chromatography [ethyl acetate:hexane]. The synthesis scheme is shown in Scheme 1.

Synthesis of 2,4-diphenyl-4H-chromene-3,4-diol; compound 1

The reaction mixture was Rota evaporated and the residue was purified using flash chromatography (7:3 Hexane: Ethyl acetate). A pure semisolid was obtained (174 mg, 55% yield). IR: ATR, υ_{max} (cm^{-1}): 3360.2 (OH stretch for alcohol). ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 6.7-7.7 (12H, m, -Ar-H), 7.7 (2H, d, -Ar-H), ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 84.1, 117.7, 118.3, 123.4, 126.2, 126.9, 127.1, 127.9, 128.9, 128.6, 129.2, 130.3, 131.5, 144.7, 150.8, 153.1

Synthesis of 4-benzyl-2-phenyl-4H-chromene-3,4-diol; compound 2

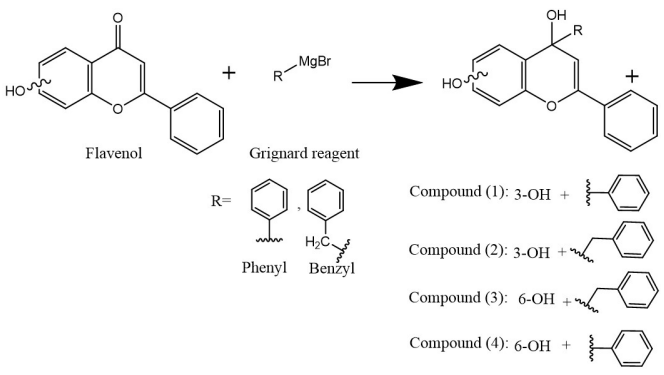
The reaction mixture was Rota evaporated and the residue was purified using flash chromatography (7:3 hexane: ethyl acetate). A pure yellow semisolid was obtained (240.7 mg, 73% yield). IR: ATR, υ_{max} (cm^{-1}): 3370.2 (OH stretch for alcohol). ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 3.03 (2H, s, CH2), 6.8-7.9 (13H, m, -Ar-H), ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 41.6, 117.4, 118.3, 121.1, 123.1, 125.9, 127.7, 127.8,, 127.9, 127.9, .5, 128.6, 128.7, 130.3, 139.4, 152.0, 150.8.

4-benzyl-2-phenyl-4H-chromene-4,6-diol; compound 3

The reaction mixture was Rota evaporated and the residue was purified using flash chromatography (9:1 Hexane: Ethyl acetate). A pure yellow semisolid was obtained (205.7 mg, 62% yield). IR: ATR, υ_{max} (cm^{-1}): 3527.7 (OH stretch for alcohol). ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 3.15 (2H, s, CH2), 5.8 (1H, s, -Ar-H), 6.6 (1H, s, -Ar-H), 6.5-7.8 (12H, m, -Ar-H), ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 47.8, 72.4, 98.1, 113.5, 115.5, 118.8, 122.5, 125.2, 125.9, 127.7, 127.9, 128.6, 135.3, 139.4, 144.6 . 151.0, 151.4.

Synthesis 2,4-diphenyl-4H-chromene-4,6-diol; compound 4

The reaction mixture was Rota evaporated and the residue was purified using flash chromatography (7:3 hexane: ethyl acetate). A pure semisolid was obtained (67.8 mg, 22% yield). IR: ATR, υ_{max} (cm^{-1}): 3339.7 (OH stretch for alcohol). ^{1}H NMR



Scheme 1. Synthesis of tertiary alcohol analogs of flavones.

(DMSO-d^6, 500 MHz) δ ppm: 5.7 (1H, s, -Ar-H), 6.8 (1H, s, -Ar-H), 6.5-7.7 (12H, m, -Ar-H), ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 76.3, 98.1, 114.1, 114.6, 119.1, 125.2, 126.2, 127.1, 127.9, 128.6, 129.2, 132.9, 135.3, 149.5, 145.7, 151.0, 151.7.

Synthesis of flavone-gallic acid derivatives

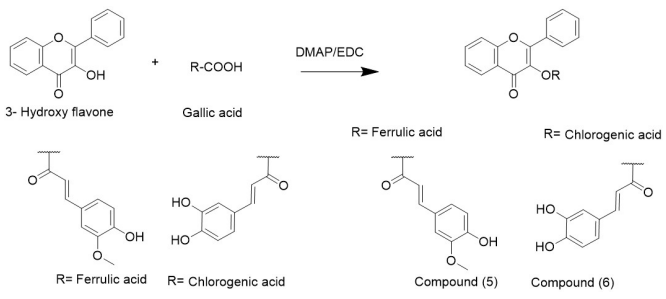
Ferrulic acid/chiogentic acid (1 mmole) was added to EDC (191.7 mg) in a round bottom both reagents were dissolved in tetrahydrofuran and dichloromethane (5: 1), the mixture was allowed to stir under argon. DMAP (122.17 mg) was added after half an hour. The mixture was allowed to stir at room temperature for overnight. On the next day 3hydroxyflavone (238 mg) was dissolved in 1 mL THF and was then added to the reaction mixture and the reaction was allowed to stir for 24 hours. The reaction was monitored by TLC (9:1 hexane: ethyl acetate). The reaction mixture was Rota evaporated and the residue was purified using flash chromatography (9:1 hexane: ethyl acetate). The reaction followed Scheme 2.

Synthesis of 4-oxo-2-phenyl-4H-chromen-3-yl(E)-3-(4-hydroxy-3-methoxy phenyl) acrylate; compound 5

A pure semisolid was obtained (90.3 mg, 22% yield). IR: ATR, υ_{max} (cm^{-1}): 3500.7 (OH stretch for alcohol). 1720 (carbonyl of ketone), 1759 (Carbonyl of ester) ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 3.8 (1H, s, -CH3), 6.7 (1H, s, -CH=), 7.7 (1H, s, -CH=), 7.9- 8.5 (12H, m, -Ar-H) ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 56.1, 111.9, 115.5, 116.1, 116.8, 121.7, 122.9, 123.4, 125.8, 127.6, 127.9, 128.6, 130.3, 133.3, 135.2, 149.1, 147.9, 153.7, 156.2, 157.2, 178.2.

Synthesis of 4-oxo-2-phenyl-4H-chromen-3-yl (E)-3-((3-(3,4-dihydroxyphenyl) acryloyl)oxy)-1,4,5-trihydroxycyclohexane-1-carboxylate; compound 6

A pure semisolid was obtained (42.6 mg, 7.5% yield). IR: ATR, υ_{max} (cm^{-1}): 3500.7 (OH stretch for alcohol). 1711 (carbonyl of ketone), 1759 (Carbonyl of ester) ^{1}H NMR (DMSO-d^6, 500 MHz) δ ppm: 2.1 (1H, 2, -CH2-), 2.2 (1H, d, -CH2-), 3.4 (1H, q, -CH-), 4.15 (1H, t, -CH2-), 4.2 (1H, q, -CH2-), 6.3 (1H, s, -CH=), 7.5 (1H, s, -CH=),, 6.7- 8.10 (12H, m, -Ar-H) ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 38.1, 38.8, 71.2, 71.6, 73.3, 75.8, 115.2, 116.1, 116.2, 117.2, 121.7, 123.2, 123.4, 125.8, 127.9, 128.0, 128.6, 130.3, 133.3, 135.2, 145.1, 145.9, 146.5, 153.7, 156.2, 166.5, 172.0, 178.2.



Scheme 2. Synthesis of flavones-gallic acid compounds.

2.4 Evaluation of anticancer activity

The anticancer activity of the compounds was studied using HeLa and CaCo-2 cell lines. The cells were cultured in a 15-cm^2 plastic culture plate in a culture growth medium (CGM) that consisted of RPMI medium supplemented with 10% fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin. Cells were maintained in the above medium at 37 °C in a humidified atmosphere containing 5% CO_2.

For subculturing, the CGM was suctioned from the 15-cm^2 culture plate. Next, the cells were washed twice using 15 mL of Ca^{2+}-free phosphate-buffered saline. Then, trypsin (1 mL) was added, and the cells were incubated for approximately 3 min at 37 °C in a humidified atmosphere containing 5% CO_2 until sufficient cells detached from the plate surface. Herein, trypsin was inactivated by 10 mL of CGM. Subsequently, the cell suspension was collected, diluted, and distributed into a 96-well plate and was left to adhere for over 24 h (Hawash et al., 2020).

HeLa and CaCo-2 cells were subcultured into a 96-well plate as explained above. After 24 h, the cells were incubated with 100 µL of synthesized compounds in triplicate with a concentration range (25 to 400 µg/mL) for 48 h. Then, MTS solution (20 µL) was added to each well, followed by an incubation period of 2 h. The absorbance was subsequently measured using a plate reader the average mean value was recorded.

2.5 In vitro test for COX-1 and COX-2 enzyme inhibitory activity

The Cyclooxygenase coenzymes (COX-1 and COX-2) inhibitory activity of the synthesized compounds was assessed using the COX (human) inhibitor screening assay kit supplied by Cayman chemicals (catalog no. 701230), Ann Arbor, MI, USA). The preparation of the reagents and the testing procedure were performed according to the manufacturer's recommendations.

Two concentrations of the inhibitors and the positive control celecoxib (10 and 50 µM) were dissolved in a minimum quantity of dimethylsulfoxide (DMSO) and were incubated with a mixture of COX-1 or COX-2 enzymes and heme in the diluted reaction buffer for 10 min at 37 °C. The reaction was initiated by adding 10 µL of arachidonic acid, followed by incubation at 37 °C for exactly 2 min. Then, the reaction was stopped by adding 30 µL of stannous chloride solution to each reaction tube, followed by incubation for 5 min at room temperature. The produced PGF2α in the samples was quantified using an enzyme-linked immunosorbent assay (ELISA). The 96-well plate was covered with a plastic film and incubated for 18 h at room temperature on an orbital shaker. After incubation, the plate was rinsed five times with the wash buffer, followed by the addition of Ellman's reagent (200 µL). Then, it was incubated for approximately 60-90 min at room temperature, until the absorbance of the well was in the range of 0.3-0.8 at 405 nm. The plate was then read using Unilab microplate reader 6000. The inhibitory percentage was measured for the different tested concentrations against the control. The IC_{50} was calculated from the concentration-inhibition response curve, and the selectivity index (SI) was calculated by dividing the IC_{50} against COX-2 by that against COX-1 (Abualhasan et al., 2020; Assali et al., 2020).

2.6 Evaluation of antioxidant activity

Antioxidant activity was assessed using the DPPH assay (Abualhasan et al., 2014; Jaradat & Abualhasan, 2015). Eight synthetic compounds were evaluated for the efficiency of scavenging free radicals. A total of 10 mg of each compound was dissolved in 100 mL of methanol to get a concentration of 100 µg/mL, and the solution was used to prepare different concentrations: 1, 2, 3, 5, 7, 10, 20, 30, 50, and 80 µg/mL. The DPPH reagent (0.002% w/v) was dissolved in methanol before being mixed with the working concentrations in a ratio of 1:1:1 (compound: DPPH: methanol). The methanol solution was used as a blank. All the solutions were incubated for 30 min in the dark at room temperature. The absorbance values were estimated using a UV-vis spectrophotometer at 517 nm. The antioxidant potential percentage of each compound was estimated according to the Equation 1:

$$I\ (\%) = ((Abs\ blank - Abs\ sample) / Abs\ blank) \times 100 \quad (1)$$

Where:

Abs blank: Absorbance value of the control reaction containing all the reagents except the synthesized compound.

Abs sample: Absorbance value of the synthesized compound.

The concentrations of the test compounds with 50% inhibition (IC_{50}) were calculated via the plot of inhibition (%) versus test compounds concentration.

2.7 Statistical analysis

Statistical Package for the Social Sciences (SPSS) software program was used to perform descriptive analysis. Analysis of variance (ANOVA) test was used to test if there was a statistical significant different between means. In all the mentioned statistical test if the p-value is less than 0.05, the null hypothesis was rejected and it was concluded that a significant difference does exist.

3 Results and discussion

3.1 Anticancer activity

Cytotoxicity assays are performed for the biological screening of many synthesized anticancer agents (Xiao et al., 2014). These assays are rapid and cheap. They include luminometric, fluorometric, dye exclusion, and colorimetric assays (Gerets et al., 2009).

The anticancer activity of all the synthesized compounds was assessed using HeLa and CaCo-2 cancer cell lines.

Some of the synthesized drugs exhibited moderate to potent anticancer activity against CaCo cells, such as compounds 2, 4, and 5. The percentage of cell growth was 3.95, 1.08, and 0.49%, respectively, at a concentration of 400 µg/mL (Table 1). Compound 3 had the most potent anticancer activity with an IC_{50} = 30.8057 µg/mL (Figure 1). Analysis of variance (ANOVA) test was done in order to test for statistical difference between the cytotoxic effect of the tested compounds. The result indicated significant difference with p value < 0.05.

The anticancer activity against HeLa cells was also potent for compounds 3, 4, and 5. The percentage of cell growth was 2.77, 3.04, and 1.49%, respectively, at a concentration of 400 µg/mL (Table 2). Compound 3 was the most potent, with IC_{50} = 30.81 µg/mL (Figure 2). Our results are comparable to

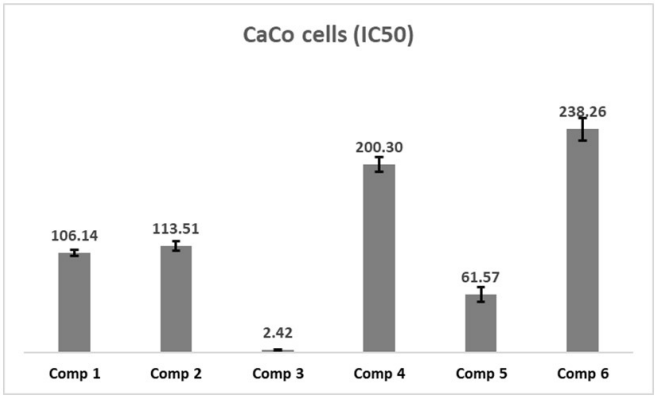


Figure 1. IC_{50} of the compounds against CaCo cells.

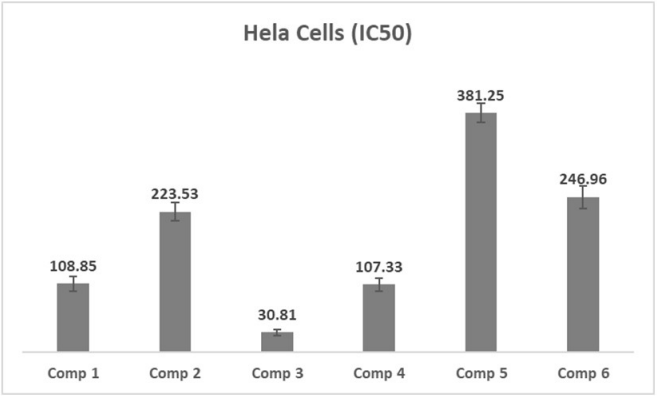


Figure 2. IC_{50} of the compounds against Hela cells.

Table 1. Percentage of living CaCo-2 cells after incubation with the synthesized compounds.

	% of living CaCo cells					
	control	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL
Comp 1	100.00	19.83 ± 1.3	18.25 ± 2.3	72.38 ± 6.3	102.94 ± 7.9	114.41 ± 4.5
Comp 2		3.95 ± 0.3	3.30 ± 1.0	81.47 ± 9.9	98.66 ± 8.3	116.82 ± 7.6
Comp 3		6.92 ± 1.5	3.29 ± 0.2	1.78 ± 0.5	0.45 ± 0.1	0.87 ± 0.3
Comp 4		1.08 ± 0.1	2.45 ± 0.6	92.27 ± 9.6	148.64 ± 9.8	120.21 ± 8.2
Comp 5		0.42 ± 0.4	0.00	26.82 ± 5.2	61.43 ± 3.2	104.16 ± 8.1
Comp 6		59.09 ± 5.3	102.87 ± 9.4	116.75 ± 8.9	120.70 ± 9.8	121.26 ± 7.4

those of our previous study that investigated the effect of *A. mannifera* on HeLa cells (Jaradat et al., 2021b).

3.2 COX inhibition activity

The inhibitory effect of the synthesized drugs was assessed against cyclooxygenase isoenzymes: COX-1 and COX-2. Figures 3-4 show the percentage inhibition of the synthesized compounds at two concentrations of 10 and 50 µg/mL against COX-1 and COX-2, respectively.

The IC_{50} was in the range of 1.58-27.04 µg/mL. However, the highest IC_{50} for COX-1 inhibition was for synthesized compound 6 (1.59 µg/mL), whereas synthesized compound 4 was the most potent inhibitor of COX-2 (IC_{50} = 6.02 µg/mL). The detailed inhibitor screening results at concentrations of 10 and 50 µg/mL are shown in Table 3. The results show that compound 4 is the most selective COX-2 inhibitor. This makes it a good target for further investigation as a promising anti-inflammatory drug. Its high selectivity for COX-2 will reduce the unwanted side effects such as peptic ulcers and bleeding that are commonly encountered with non-selective non-steroidal anti-inflammatory drugs (NSAIDs) (Meek et al., 2010). However, the result less potent then Celecoxib which was run as a positive control with an IC_{50} = 0.0152 (µg/mL).

3.3 Antioxidant activity

The antioxidant potential of the synthesized compounds was assessed using DPPH. The calculated percentage inhibition is shown in Figure 5. The results showed variable antioxidant activity. In general, moderate antioxidant activity was observed for most of the synthesized compounds when compared to Trolox antioxidant activity.

The results of the free radical scavenging activity test showed that all the drugs had moderate antioxidant activity compared to the positive control Trolox, except for compound 4 which showed potent antioxidant activity (Table 4).

We previously showed the antioxidant activity of four fractions from *E. alata* fruits which have flavonoids. The aqueous crude extract had an IC_{50} value of 15.25 ± 0.30 µg/mL. The synthesized compound 4 exhibited a more potent antioxidant activity which

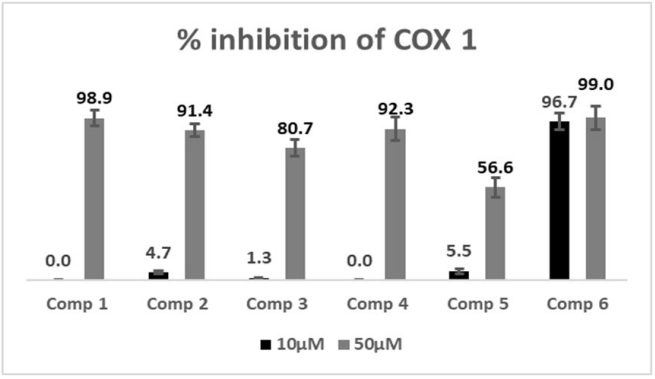


Figure 3. Percentage inhibition of the synthesized compounds against COX-1.

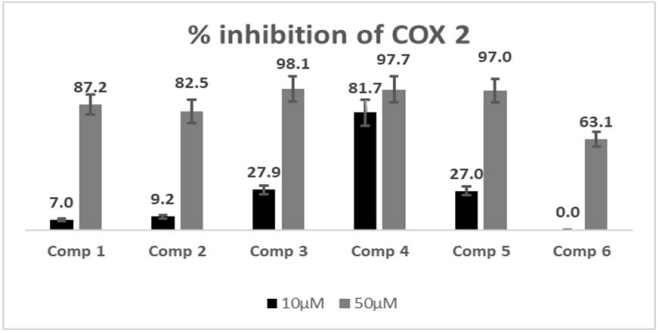


Figure 4. Percentage inhibition of the synthesized compounds against COX-2.

Table 2. Percentage of living Hela cells after incubation with the synthesized compounds.

	% of living cells of Hela Cells					
	control	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL
Comp 1	100.00	10.27	13.14	60.95	86.81	96.96
Comp 2		10.27	42.51	69.91	89.33	95.45
Comp 3		2.77	1.21	8.41	4.88	87.31
Comp 4		3.04	8.12	61.62	70.60	105.57
Comp 5		1.48	17.62	28.68	39.07	49.42
Comp 6		64.27	94.09	107.87	105.25	107.15

Table 3. The IC_{50} values of the synthesized compounds against COX-1 and COX-2 with calculated selectivity for COX-2.

Comopound	(IC_{50}) for COX-1 (µg/mL)	(IC_{50}) for COX-2 (µg/mL)	Selectivity for COX-2
Comp 1	19.07 ± 0.22	15.42 ± 0.23	1.24
Comp 2	16.49 ± 0.52	14.83 ± 0.34	1.11
Comp 3	18.32 ± 0.63	14.42 ± 0.34	1.27
Comp 4	27.40 ± 1.11	6.02 ± 0.33	4.55
Comp 5	15.26 ± 0.52	14.56 ± 0.23	1.05
Comp 6	1.58 ± 0.11	27.63 ± 0.67	0.06

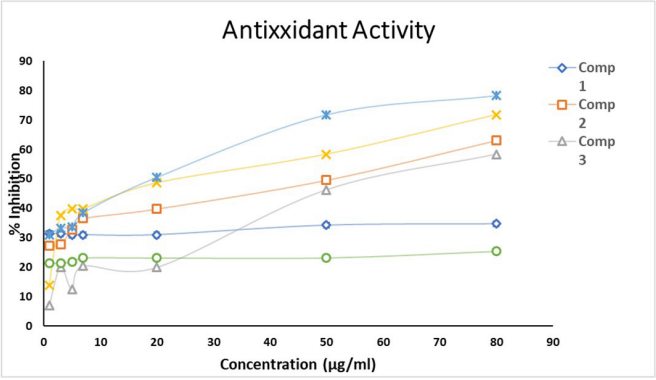


Figure 5. % inhibition of DPPH of the synthesized compounds.

Table 4. Antioxidant IC_{50} of the synthesized compounds.

Compound Number	IC_{50} (µg/mL)	
	Synthesized Compounds	Control
1	39.12 ± 4	2.23 ± 0.92
2	47.33 ± 4	
3	60.78 ± 5	
4	3.53 ± 0.1	
5	30.81 ± 3	
6	66.09 ± 4	

was comparable to that of Trolox (IC_{50} = 2.23 ± 0.92 µg/mL) (Jaradat et al., 2021a). Statistical test using ANOVA test indicated no significant difference between the compounds (P > 0.05).

4 Conclusion

We successfully synthesized a group of novel compounds that are derivatives of flavones, as well as flavones combined with gallic acid. The bioactivity of these compounds was assessed, such as their anticancer, antioxidant, and anti-inflammatory effects. Compound 3 showed very potent anticancer activity, while compound 4 showed highly selective COX-2 inhibitory activity, rendering it an effective anti-inflammatory drug. Moreover, compound 4 showed potent antioxidant activity. Future studies should aim at investigating and confirming the efficacy of the in vitro tested compounds through an in vivo study. More synthesis of promising compounds is required in order come up with structure-activity relationship.

Availability of data and material

The datasets acquired and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by the deanship of scientific research at An Najah National University, under fund identification number [ANNU-2021-Sc0011].

Acknowledgements

The authors would like to acknowledge the department of pharmacy, An-Najah National University, for facilitating the accomplishment of the current work.

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