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Article

# Biological Evaluation of Xanthene and Thioxanthene Derivatives as Antioxidant, Anticancer, and COX Inhibitors

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**ABSTRACT:** Xanthene and thioxanthene analogues have been investigated for their potential as anticancer and anti-inflammatory agents. Additionally, cysteine analogues have been found to possess antioxidant, anti-inflammatory, and anticancer activities due to their role in cellular redox balance, scavenging of free radicals, and involvement in nucleophilic reactions and enzyme binding sites. In this study, we synthesized a library of tertiary alcohols derived from xanthene and thioxanthene, and further, some of these compounds were coupled with cysteine. The objective of this research was to explore the potential anticancer, antioxidant, and anti-inflammatory activities of the synthesized compounds. The synthesized compounds were subjected to test for anticancer, antioxidant, and anti-inflammatory activities. Results indicated that compound **3** exhibited excellent inhibition activity ($IC_{50}$ = 9.6 ± 1.1 nM) against colon cancer cells (Caco-2), while compound **2** showed good inhibition activity ($IC_{50}$ = 161.3 ± 41 nM) against hepatocellular carcinoma (Hep G2) cells. Compound **4** demonstrated potent antioxidant inhibition activity ($IC_{50}$ = 15.44 ± 6 nM), and compound **7** exhibited potent anti-inflammatory activity with cyclooxygenase-2 (COX-2) inhibition $IC_{50}$ (4.37 ± 0.78 nM) and high selectivity for COX-2 (3.83). In conclusion, certain synthesized compounds displayed promising anticancer activity and anti-inflammatory effects. Nevertheless, additional research is necessary to create more analogues, develop a more distinct comprehension of the structure−activity relationship (SAR), and perform in vivo experiments to evaluate the pharmacokinetic and pharmacodynamic characteristics of the compounds under examination. Such research may pave the way for the development of novel therapeutic agents with potential applications in cancer and inflammatory diseases.

## 1. INTRODUCTION

Cancer is a significant health concern globally and is one of the leading causes of death. As of 2020, it has accounted for nearly 10 million deaths worldwide.[1] The main treatment options for cancer include chemotherapy, radiation therapy, and surgery.[2−6] These treatment modalities are used either alone or in combination, depending on the type and stage of cancer as well as individual patient factors. Chemotherapy is one of the most widely used methods for treating cancer patients. The choice of cytotoxic drug used in chemotherapy depends on several factors, including the type of tumor, histologic grade (the appearance of cancer cells under a microscope), stage of disease, and the patient's ability to tolerate the side effects of the treatments.[7] Chemotherapy does have significant limitations, which have crucial considerations in cancer treatment.[8] Chemotherapy resistance can lead to disease progression and a reduced treatment response. Chemotherapy may cause toxic side effects, including bone marrow suppression, gastrointestinal problems (nausea, vomiting, diarrhea), hair loss (alopecia), and neutropenia.[1,2,7,9]

Selective cytotoxic agents are designed to target cancer cells more specifically, potentially reducing the impact on healthy cells and minimizing side effects. One such agent is S-trityl-L-cysteine (STLC). It is a selective cytotoxic agent that has been studied for its potential in cancer treatment.[10] This compound has utilized cysteine in its structure along with triaromatic rings and was identified as an ATP-noncompetitive and potent reversible inhibitor of human mitotic kinesin Eg5, which works as an antimitotic chemotherapeutic agent.[11−18] However, STLC development was difficult because of the pharmacokinetic problems.[12,19] STLC has poor water solubility and poor permeability, which affects the bioavailability; this is due to the amphiphilic character of STLC.[16,19]

Free radicals are chemically reactive molecules that have unpaired electrons. They can be generated from both internal sources, such as cellular metabolism, and external sources, such as exposure to environmental toxins or ultraviolet (UV) radiation.[20] These compounds at high concentrations become harmful and cause oxidative damage to various cellular components, including lipids, proteins, and DNA.[20−22] Oxidative stress occurs when the production of free radicals overwhelms the body's ability to neutralize them with antioxidants. This imbalance leads to an excess of oxidative damage and is associated with various disease conditions, including cancer.[21,23,24] Antioxidant compounds counteract the harmful effects of free radicals by scavenging free radicals, absorbing UV radiation, binding metal ions that catalyze

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oxidative reactions, and converting hydroperoxides into nonradical species.[25] Cysteine, an amino acid with a thiol group (−SH), is believed to act as an antioxidant by donating a hydrogen atom to its thiol group. This donation helps neutralize free radicals and other reactive species, thus protecting cells from oxidative damage.[26−28] Thus, antioxidant compounds, including cysteine, play a vital role in combating oxidative stress and reducing the risk of cancer by neutralizing harmful reactive oxygens and free radicals.

Chronic inflammatory diseases are the leading cause of death. According to the World Health Organization (WHO), chronic diseases pose the biggest risk to human health.[29] Inflammation is characterized by the accumulation of fluids and leukocytes leading to edema and pain.[30] The inflammatory response mediated by different physiological and immunological mediators that play a role in acute and chronic inflammation, such as prostaglandins.[31,32] When arachidonic acid is catalyzed by the cyclooxygenase enzyme, one of the prostaglandins (PGE2) is formed, which is the principal mediator of inflammation. There are two isoforms of the cyclooxygenase enzyme: COX-1 and COX-2.[33] NSAIDs are groups of compounds that have an effective role in relieving pain, inflammation, and fever.[34,35] The mechanism of action by which NSAIDs alleviate pain and their role in the inflammation process is made by the inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase enzyme. However, their chronic use may cause gastrointestinal tract (GIT) ulceration, bleeding, and renal injury.[30,36−38] On the other hand, compared to traditional NSAIDs, specific inhibition of the inducible COX-2 isozyme will offer a helpful treatment approach with less adverse effects. In this context, many selective COX-2 inhibitors (COXIBs), including rofecoxib, celecoxib, valdecoxib, and etoricoxib, were developed and commercialized.[39] It was discovered by examining the structures of the most selective COX-2 inhibitors that the carbocyclic or heterocyclic cores contain diaryl substitution. Furthermore, COX-2 selectivity increased as a result of hydrophobic contacts with additional hydrophobic area and polar interactions with a secondary polar pocket that are present in COX-2 isozyme, as shown by structure−activity relationship (SAR) analyses of COXIBs.[40,41]

L-cysteine is a nutritionally semiessential amino acid.[42] It plays an important role in many processes, such as protein folding stability and trafficking, assembly, biosynthesis of coenzyme A, detoxification of heavy metals, and redox balance.[43,44] Inside cells, L-cysteine is the prevailing form due to the highly reducing conditions.[45] The thiol group present in the structure of cysteine makes it a unique amino acid, which can undergo a variety of different nucleophilic reactions and increase its affinity to enzyme binding sites of metals, such as zinc or iron.[46,47] Xanthene and thioxanthene are tricyclic structures; many derivatives of these structures showed anticancer and biological activities.[48−50]

To the best of our knowledge, none of the produced analogues with a cysteine-coupled xanthene−thioxanthene core have been tested for anticancer, antioxidant, or anti-inflammatory effects. The objective of this research is to synthesize a collection of xanthene and thioxanthene analogues coupled with cysteine and evaluate their biological activity. The anticancer activity of the synthesized analogues was tested against HeLa cells, hepatic cancer cells (Hep G2), and the colon cancer cell line (Caco-2). Furthermore, the antioxidant and cyclooxygenase (COX) inhibitory properties of the produced analogues were investigated.

## 2. MATERIALS AND METHODS

**2.1. Reagents and Materials.** All reagents were obtained commercially and used without further purification. Xanthone (catalog no. A14812), thioxanthone (catalog no. A 18131), boron trifluoride diethyl etherate, and 98+% (catalog no. A15275) were purchased from Alfa Aesar Company, England. Benzyl magnesium chloride solution (catalog no. 302759), phenyl magnesium chloride solution (catalog no. 224448), and L-cysteine (catalog no. 168149) were purchased from Sigma-Aldrich, Germany. Acetone, methanol, dichloromethane, hexane, and ethyl acetate were purchased from C.S. Company, Haifa. Diethyl ether (catalog #38132) was purchased from Merck Millipore, and tetrahydrofuran (THF) solvent (catalog #487308) was purchased from Carlo Erba Company, MI, Italy. Sodium sulfate, ammonium chloride, and sodium bicarbonate were purchased from C.S. Company, Haifa. Cayman COX (human) Inhibitor Screening Assay Kit (Item #701230) was used. RPMI 1640 culture medium, trypsin, glutamine, fetal calf serum, and other reagents will be of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (catalog no. 224448) was purchased from Sigma-Aldrich, Germany.

Silica gel (Merck, 230−400 mesh) was used for flash chromatography. Chromatography columns were eluted with positive air pressure. For evaporation of solvents, a Rota Vapor (Heidolph) was used. NMR analysis was measured by a Bruker Avance 500 spectrometer at Jordan University. Chemical shifts were reported in parts per million, and coupling constants were reported in Hz. An Accumax Variable micropipette, U.K., was used for pipetting. A Unilab microplate reader 6000 was used to read the Cayman ELISA kit.

**2.2. Chemical Synthesis and Characterization of the Products.** The synthetic procedures, enzyme screening, and anticancer activity tests were conducted at An-Najah National University laboratories. NMR measurements, on the other hand, were carried out at the University of Jordan using a Bruker Avance 400 instrument. The compounds were synthesized in two stages. First, the tertiary alcohols were synthesized. Then, these alcohols were coupled with L-cysteine.[10]

*2.2.1. General Synthesis of Tertiary Alcohols.* The required ketone (1 mmol) and Grignard reagent (2 mmol) were mixed in a round-bottom flask under a nitrogen atmosphere. This reaction was conducted at 0 °C. The mixture was agitated for 24 h. After the reaction period, the mixture was quenched by adding 10 mL of ammonium chloride saturate. The quenched mixture was then extracted with three 20 mL portions of ethyl acetate. The ethyl acetate extract was desiccated by using sodium sulfate. After desiccation, the solvent was evaporated under reduced pressure. The residue obtained after solvent evaporation was then subjected to purification by using silica gel chromatography. Silica gel was used as a stationary phase, and a mixture of ethyl acetate and hexane in a 1:1 ratio was used as the mobile phase. The purified fractions containing the desired compound were collected.[51]

*2.2.1.1. Synthesis of 9-Benzyl-9H-xanthen-9-ol: Compound 1.* To obtain compound **1**, 9*H*-xanthen-9-one (196.21 mg, 1 mmol) was reacted with benzyl magnesium chloride (292.7 μL, 2 mmol) and DCM (5 mL). A pure yellow powder was obtained (180 mg, 60.3% yield). $R_f$: 0.5 (ethyl acetate/hexane (1:2)) M.P.: 200 − 202 °C. IR: ATR, $\upsilon_{max}$


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($cm^{-1}$): 3367.2 (OH stretch for alcohol). $^1$H NMR (500 MHz): $\delta$ ($CD_3OD$): 3.15 (2H,s,−C**H**$_2$) 6.25−7.75; (13H, m, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 73.84, 115.01−129.26 (15C, Ar); 137.24; 151.67.

*2.2.1.2. Synthesis of 9-Benzyl-9H-thioxanthen-9-ol: Compound* ***2****.* To obtain compound **2**, 9*H*-thioxanthen-9-one (212 mg, 1 mmol) was reacted with with benzyl magnesium chloride (292.7 μL, 2 mmol), DCM (5 mL). A pure yellow powder was obtained (155 mg, 55.5% yield). $R_f$: 0.55 (ethyl acetate/hexane (1:2)), M.P.: 185 −188 °C. IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3321.2 (OH stretch for alcohol). $^1$H NMR (500 MHz): $\delta$ ($CDCl_3$): 2.95 (1H, s, −O**H**); 3.30 (2H, s, −C**H**$_2$); 6.66 (2H, d, $J$ = 8.0 Hz); 6.97 (3H, m, Ar); 7.15 (4H, m, Ar); 7.37 (2H, d, $J$ = 7.6 Hz, Ar); 7.57 (2H, d, $J$ = 7.6 Hz, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 43.37; 74.64; 125.64−126.92 (15C, Ar); 136.33; 140.34.

*2.2.1.3. Synthesis of 9-Phenyl-9H-thioxanthen-9-ol: Compound* ***3****.* To obtain compound **3**, 9*H*-xanthen-9-one (196 mg, 1 mmol) was reacted with phenyl magnesium chloride (268.35 μL, 2 mmol) and DCM (5 mL). A pale yellow powder was obtained (150 mg, 50.6% yield). $R_f$: 0.4 (ethyl acetate/hexane (1:2)) M.P.: 190 −192 °C. IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3226.9 (OH stretch for alcohol). $^1$H NMR (500 MHz): $\delta$ ($CDCl_3$): 2.59 (1H, s, −O**H**); 7.03 (2H, t, $J$ = 8.0 Hz, Ar); 7.18 (3H, m, Ar); 7.27−7.40 (8H, m, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 68.64; 115.78−128.76 (15C, Ar); 149.07−149.68.

*2.2.1.4. Synthesis of 9-Phenyl-9H-xanthen-9-ol: Compound* ***4****.* To obtain compound **4**, 9*H*-thioxanthen-9-one (212 mg, 1 mmol) was reacted with phenyl magnesium chloride (268.35 μL, 2 mmol) andTHF (5 mL). A pure yellow powder was obtained (90 mg, 42.4% yield). $R_f$: 0.5 (ethyl acetate/hexane (1:2)) M.P.: 205 − 208 °C. IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3549.15 (OH stretch for alcohol).$^1$H NMR (500 MHz): $\delta$ ($CDCl_3$): 2.71 (1H, s, −O**H**); 6.98 (2H, m, Ar); 7.17 (3H, m, Ar); 7.29 (2H, m, Ar); 7.42 (4H, m, Ar); 8.04 (2H, d, $J$ = 8.4 Hz, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 75.32; 126.01−130.28 (15C, Ar); 140.64; 144.27.

*2.2.2. General Synthesis of Xanthene and Thioxanthene Analogues.* In a reaction vessel, 0.5 mL of acetic acid was used as the solvent, and L-cysteine (1 mmol) and the corresponding tertiary alcohol (1 mmol) were dissolved in the solution. Boron trifluoride diethyl etherate, a catalyst, was added drop by drop to the reaction mixture while maintaining a temperature of 0 °C. The reaction mixture was agitated for two h at 0 °C to allow for the reaction to take place. The progress of the reaction was monitored by using thin-layer chromatography (TLC). The reaction was stopped by adding 1.5 mL of sodium acetate solution (10% concentration) and 1.5 mL of a water mixture. The resultant precipitate that formed after stopping the reaction was filtered from the reaction mixture. The precipitate was then rinsed with water. The filtered precipitate was further rinsed with diethyl ether, and it was then dried in a vacuum oven at 40 °C for 24 h. The final product, the L-cysteine derivatives, was obtained after the drying process and collected for further characterization and analysis. The proton and carbon assignment atoms of the synthesized compounds and their structure are illustrated in the Supporting File.

*2.2.2.1. Synthesis of S-(9-benzyl-9H-xanthen-9-yl)-L-cysteine: Compound* ***5****.* To obtain compound **5**, L-cysteine (121 mg,0.48 mmol) was reacted with 9-benzyl-9*H*-thioxanthen-9-ol (compound **2**, 288 mg, 1 mmol) and boron trifluoride diethyl etherate (207 μL,1.68 mmol). A pure white powder was obtained (78 mg, 40.3% yield). $R_f$: 0.3(DCM/MeOH (9:1)), M.P.: 230 − 233 °C IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3388.15 (OH stretch for carboxylic acid), 1630 (C═O stretch). $^1$H NMR (500 MHz): $\delta$ (DMSO-$d_6$): 2.29 (1H, dd, $J$ = 12.8, $J$ = 10.0 Hz, − CHaHb); 2.56 (1H, dd, $J$ = 12.8, $J$ = 4.4 Hz, −CHaHb); 2.79 (1H, dd, $J$ = 9.6, $J$ = 4.4 Hz); 3.40 (2H, s, −$CH_2$); 6.30 (2H, d, $J$ = 7.6 Hz); 6.83−7.28 (9H, m, Ar); 7.94 (1H, d, $J$ = 7.6 Hz); 8.02 (1H, d, $J$ = 7.6 Hz). The protons of $NH_2$ and −OH were too broad to be observed. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 32.24; 51.39; 53.49; 53.84; 116.01−130.26 (15C, Ar); 136.24; 150.67; 168.46.

*2.2.2.2. Synthesis of S-(9-phenyl-9H-xanthen-9-yl)-L-cysteine: Compound* ***6****.* To obtain compound **6**, L-cysteine (121 mg,1 mmol) was reacted with 9-phenyl-9*H*-xanthen-9-ol (compound **4**, 247.3 mg, 1 mmol) and boron trifluoride diethyl etherate (207 μL, 1.68 mmol). A pure yellow powder was obtained (90 mg, 47.4% yield). $R_f$: 0.1(DCM/MeOH (9:1)), M.P.: 235 − 238 °C. IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3370.5 (OH stretch for carboxylic acid), 1620.06 (C═O stretch). $^1$H NMR (500 MHz): $\delta$ (DMSO-$d_6$): 2.38 (1H, dd, $J$ = 12.0), $J$ = 10.0 Hz, −CHaHb; 2.63 (1H, dd, $J$ = 12.0 Hz), $J$ = 4.0 Hz, −CHaHb; 2.83 (1H, dd, $J$ = 9.6), $J$ = 4.0 Hz; 7.06 (2H, m, Ar); 7.17−7.38 (11H, m, Ar). The protons of $NH_2$ and −OH were too broad to be observed. $^{13}$C NMR (DMSO-$d_6$) $\delta$ ppm: 32.29; 53.08; 55.15; 116.01−130.04; 145.32; 150.25; 167.86.

*2.2.2.3. Synthesis of S-(9-phenyl-9H-thioxanthen-9-yl)-L-cysteine: Compound* ***7****.* To obtain compound **7**, L-cysteine (0.121 g, 1 mmol) was reacted with 9-phenyl-9*H*-thioxanthen-9-ol (compound **3**, 290.4 mg, 1 mmol) and boron trifluoride diethyl etherate (207 μL, 1.68 mmol). A pure yellow powder was obtained (120 mg, 55.7% yield). $R_f$: 0.3 (DCM/MeOH (9:1)). M.P.: 250−252 °C. IR: ATR, $\upsilon_{max}$ ($cm^{-1}$): 3370.5 (OH stretch for carboxylic acid), 1620.95 (C═O stretch). $^1$H NMR (500 MHz): $\delta$(DMSO-$d_6$): 2.48 (1H, dd, $J$ = 12.8 Hz) $J$ = 8.8 Hz, −CHaHb; 2.60 (1H, dd, $J$ = 12.8), $J$ = 4.8 Hz, −CHaHb; 2.98 (1H, dd, $J$ = 8.8), $J$ = 4.8 Hz; 7.21−7.36 (11H, m, Ar); 7.46 (2H, d, $J$ = 7.6 Hz, Ar). The protons of $NH_2$ and −OH were too broad to be observed. $^{13}$C NMR: (DMSO-$d_6$) $\delta$ ppm: 33.51; 53.12; 62.35; 126.59−132.09 (15C, Ar); 136.42; 142.44; 167.89.

**2.3. Anticancer Test.** The anticancer activity of the compounds was evaluated against various cancer cell lines, including hepatocellular carcinoma (Hep G2), colon carcinoma (Caco-2), and cervical adenocarcinoma (HeLa) cells.

The cancer cells were cultured in 15 cm plastic culture plates containing culture growth medium (CGM). The CGM consisted of PRMI medium supplemented with 10% fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin. The plates were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide ($CO_2$) to support cell growth.

When the cells reached confluency in the 15 cm culture plates, the CGM was aspirated. The cells were then rinsed twice with 15 mL of phosphate-buffered saline (PBS) devoid of $Ca^{2+}$. After that, 1 mL of trypsin was added to the cells, and the plate was incubated for approximately 3 min at 37 °C in a humidified atmosphere containing 5% $CO_2$. To inactivate the trypsin, 10 mL of CGM was added to the plate. The cell suspension was then collected and diluted. The diluted cell suspension was disseminated into a 96-well plate and left to adhere for 24 h. After the 24 h adherence period, the cells in the 96-well plate were incubated with 100 μL of a concentration range (100−20,000 nM) dissolved in 1% DMSO solvent for all of the compounds under investigation,


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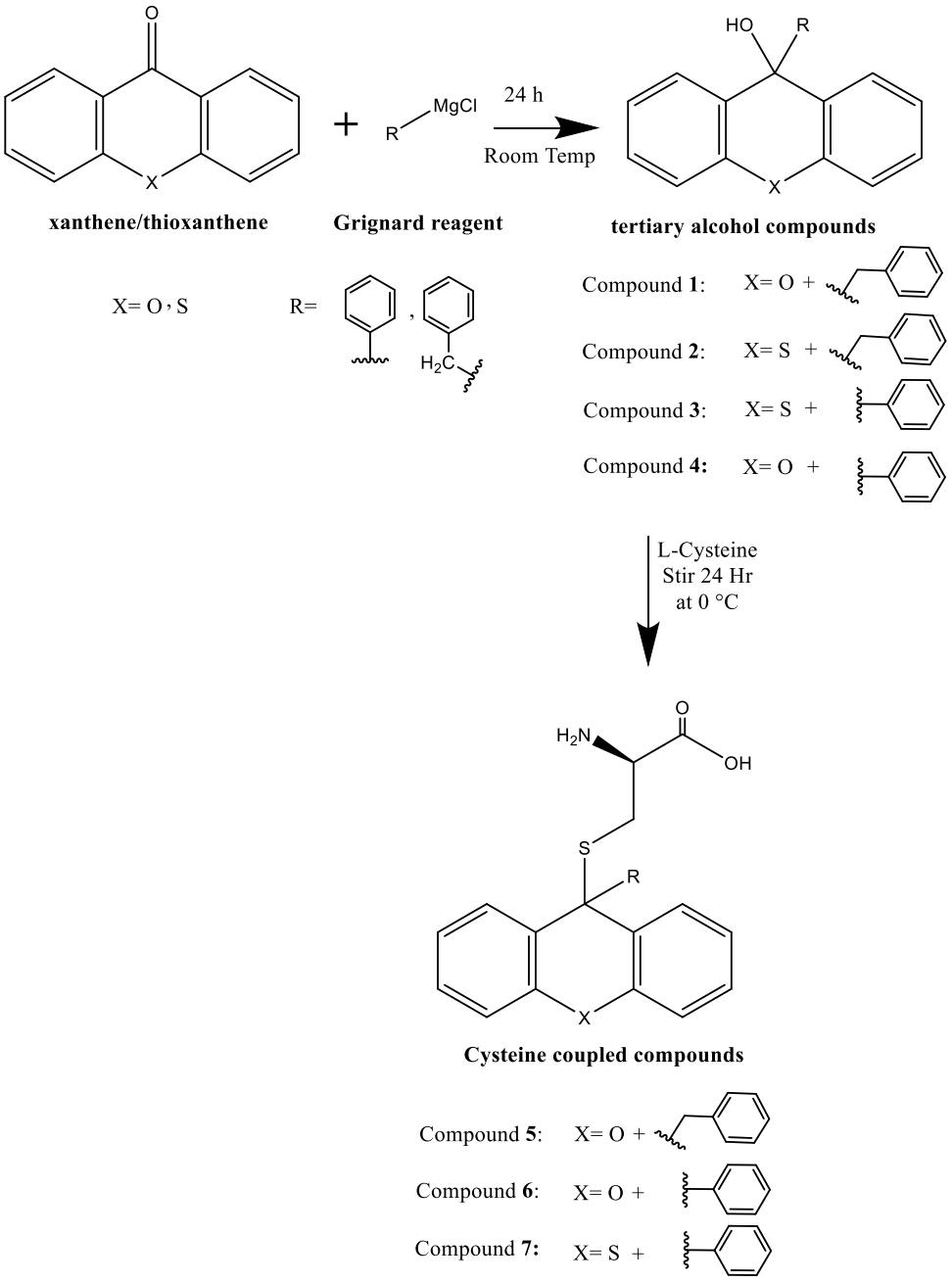
**Scheme 1. Chemical Synthesis of Tertiary Alcohol and Cysteine-Coupled Compounds**




as well as a positive control, doxorubicin. The compounds were then incubated with the cells for 48 h.

After the incubation period, 20 $\mu$L of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) solution was added to each well. The plate was then incubated for 2 h, and then, the absorbance of each well was measured using a plate reader.[52]

**2.4. Antioxidant Test.** Antioxidant activity was examined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[53] DPPH is a widely used compound in assays for measuring the in vitro antioxidant activity. The DPPH assay is relatively simple and straightforward to perform. This simplicity makes them accessible and convenient for researchers. Moreover, the DPPH assay can be applied to a wide range of samples, including plant extracts, natural compounds, and synthesized molecules. The DPPH assay has been widely studied, validated, and referenced in the scientific literature. Its reliability and well-established protocols make it a trusted method for measuring antioxidant activity. Seven synthetic compounds were tested for the efficiency of scavenging free radicals matched along with the positive control (Trolox); 10 mg of each compound was dissolved in 100 mL of methanol in order to get a concentration of 100 $\mu$g/mL, and the solution was used to prepare different concentrations of 1, 2, 3, 5, 7, 10, 20, 30, 50, and 80 $\mu$g/mL. The DPPH reagent (0.002% w/v) was dissolved in methanol before being mixed with working concentrations in ratios of 1:1:1 (compound/DPPH/methanol). The methanol solution was used as a blank. All of the solutions were incubated for 30 min in the dark at room temperature. The absorbance values were estimated by using a UV−vis spectrophotometer at a wavelength of 517 nm. The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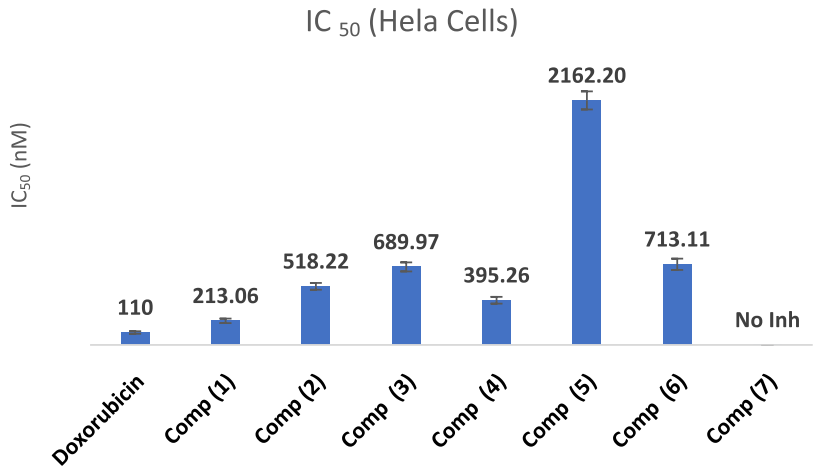


**Figure 1.** $IC_{50}$ of inhibition activity of the synthesized compounds in the concentration range of (100−20,000 nM) against HeLa cells.

antioxidant potential percentage of inhibition of each compound was estimated according to this equation

$$\% \text{ inhibition} = ((\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}) \times 100$$

where, $\text{Abs}_{\text{blank}}$ is the absorbance value of the control reaction containing all reagents except for the synthesized compound and $\text{Abs}_{\text{sample}}$ is the absorbance value of the synthesized compound.

Test compound concentrations that giving 50% inhibitions ($IC_{50}$) were calculated via the plot of inhibition (%) against the test compound concentration applying the generated multiple regression best-fit line.[51]

**2.5. Cyclooxygenase Inhibition Activity of COX-1 and COX-2 Enzymes.** The COX-1 and COX-2 inhibitory activity of our synthesized compounds and the positive control (celecoxib) were tested on the COX (human) Inhibitor Screening Assay Kit supplied by Cayman Chemicals (catalog no. 701230, Ann Arbor, MI). The preparation of the reagents and the testing procedure were performed according to the manufacturer's recommendations.

Two concentrations of the inhibitors and positive control celecoxib (0.1 and 2 $\mu$M) were dissolved in a minimum quantity of dimethyl sulfoxide (DMSO) and were incubated with a mixture of COX-1 or COX-2 enzyme, heme in the diluted reaction buffer for 10 min at 37 °C. The reaction was initiated by adding 10 $\mu$L of arachidonic acid, followed by incubation at 37 °C for exactly two min. Then, the reaction was stopped by adding 30 $\mu$L of stannous chloride solution to each reaction tube, followed by incubation for 5 min at room temperature. The produced PGF2a in the samples by COX reactions was quantified via 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 washed buffer, followed by the addition of Ellman's reagent (200 $\mu$L) and incubated for about 60−90 min at room temperature until the absorbance of the Bo well is in the range 0.3−0.8 at 405 nm. The plate was then read by an ELISA plate reader and a 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 $IC_{50}$ COX-1 by the $IC_{50}$ COX-2.

**2.6. Statistical Analysis.** All of the data of cytotoxicity activity were presented as the average of triplicate analysis. The outcomes were presented as means ± standard deviation (SD).

## 3. RESULTS AND DISCUSSION

**3.1. Chemistry.** All of the compounds were synthesized as outlined in Scheme 1. The xanthone and thioxanthone reagents were used in the reaction to synthesized tertiary alcohols, and cysteine was coupled to the synthesized tertiary phenyl and benzyl alcohols. The rings were incorporated as they were proposed to enhance the interaction pattern in terms of hydrophobic and $\pi-\pi$ interactions. Furthermore, the cysteine moiety was coupled to some of the compounds to actively contribute to cancer metabolic remodeling through redox control and to enhance the antioxidant effect of the synthesized structure.[54,55]

The synthesis strategy to form the final products involved Grignard reagents and BF3 reagents as an alkylating agent and as Lewis acid aiding the coupling of cysteine agents, respectively. The products were purified using solvent systems (*n*-hexane/ethyl acetate). Cysteine-coupled compounds showed sharp bands for carbonyl around 1600 $cm^{-1}$, while the tertiary alcohol showed OH stretch around 3500 $cm^{-1}$. The $^{1}H$ NMR peaks confirmed the synthesis of these products; a single peak of one proton for OH in the range of 2.7−2.9 ppm was observed for all tertiary alcohol compounds. Multiple signals in the aromatic area were observed. The $^{13}C$ NMR spectrum showed a C signal of carbonyl around 168 ppm.

**3.2. Anticancer Activity.** The anticancer activities were tested against different cancer cell lines, including HeLa cells, hepatic cancer cells (Hep G2), and colon cancer (Caco-2) cell lines. Some of the synthesized drugs showed potent cytotoxicity against specific cancer cell lines.

Compound **1** showed excellent inhibition activity with an $IC_{50}$ of 213.06 nM against HeLa cells.

At a higher concentration of 0.2 $\mu$M, compound **4** and compound **1** exhibited high inhibition activity against HeLa cells (98 and 96% inhibition, respectively). Compound **7** showed no activity against HeLa cells. Tertiary alcohols (compounds without cysteine coupling compounds **1** and **2**) demonstrated better activity compared to compounds coupled with cysteine (compounds **5** and **6**). Compound **1** had the best activity with an $IC_{50}$ of 213.06 ± 11 nM, which was approximately half the potency of the positive control


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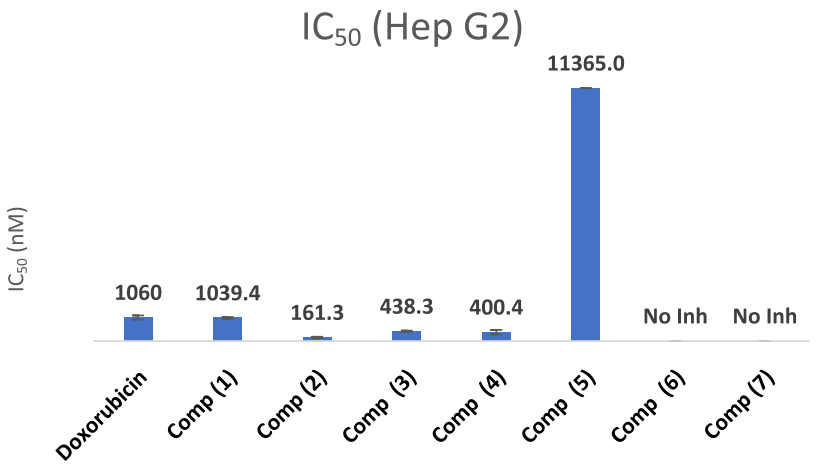


**Figure 2.** $IC_{50}$ of inhibition activity of the synthesized compounds in the concentration range of (100−20,000 nM) against Hep G2 cells.

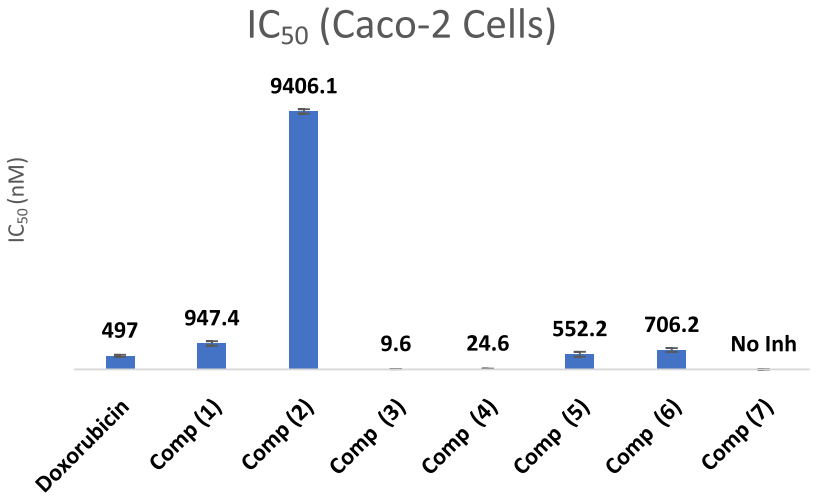


**Figure 3.** $IC_{50}$ of inhibition activity of the synthesized compounds in the concentration range of (100−20,000 nM) against Caco-2 cells.

**Table 1. Antioxidant Activity for the Synthesized Drugs**

| | percentage inhibition of DPPH | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| conc $\mu$g/mL | trolox | comp (**1**) | comp (**2**) | comp (**3**) | comp (**4**) | comp (**5**) | comp (**6**) | comp (**7**) |
| **1** | 37 ± 2.21 | 0.31 ± 0.02 | 0.26 ± 0.01 | 0.14 ± 0.02 | 10 ± 0.01 | 5.0 ± 0.01 | 18.0 ± 1.02 | 22.0 ± 3.31 |
| **2** | 54 ± 3.42 | 0.36 ± 0.02 | 0.26 ± 0.03 | 0.15 ± 0.01 | 16.0 ± 0.02 | 6.0 ± 0.02 | 18.0 ± 2.03 | 22.0 ± 3.11 |
| **3** | 81 ± 4.25 | 0.36 ± 0.01 | 0.33 ± 0.04 | 0.15 ± 0.03 | 27.0 ± 0.01 | 7.0 ± 0.01 | 22.0 ± 1.01 | 22.0 ± 2.22 |
| **5** | 86 ± 5.33 | 0.31 ± 0.01 | 0.33 ± 0.01 | 0.29 ± 0.01 | 33.0 ± 1.21 | 7.0 ± 0.51 | 28.0 ± 0.01 | 29.0 ± 4.22 |
| **7** | 91 ± 7.52 | 36.40 ± 4.11 | 26.00 ± 7.20 | 18.20 ± 1.40 | 33.0 ± 2.13 | 7.0 ± 0.67 | 28.0 ± 0.01 | 22.0 ± 1.22 |
| **10** | 92 ± 10.71 | 36.40 ± 3.31 | 27.00 ± 1.03 | 18.00 ± 2.37 | 35.0 ± 2.31 | 7.0 ± 0.35 | 30.0 ± 0.01 | 26.0 ± 3.44 |
| **20** | 93 ± 13.90 | 40.20 ± 5.21 | 28.00 ± 2.14 | 15.60 ± 2.01 | 35.0 ± 3.31 | 11.4 ± 1.01 | 30.0 ± 0.01 | 23.0 ± 1.11 |
| **30** | 94 ± 10.89 | 35.50 ± 1.01 | 29.00 ± 1.15 | 19.90 ± 1.0 | 39.0 ± 5.51 | 11.4 ± 2.10 | 30.0 ± 0.01 | 23.0 ± 1.21 |
| **50** | 94.5 ± 12.64 | 35.10 ± 3.31 | 32.00 ± 3.75 | 20.00 ± 3.12 | 39.4 ± 2.44 | 12.0 ± 1.33 | 30.0 ± 0.01 | 23.0 ± 3.10 |

anticancer drug doxorubicin ($IC_{50}$ = 110 ± 15 nM) when tested against the same cell line. The calculated $IC_{50}$ values of all of the synthesized compounds are illustrated in Figure 1[56]

The test results show that the compounds tested for their inhibition activity against Hep G2 cells produced variable results. Specifically, compound **4** and compound **2** showed complete inhibition (100%) at a concentration of 0.2 $\mu$M. On the other hand, compound **6** and compound **7** did not exhibit any inhibition. Further details about the inhibitory activity can be found in Figure 2. The positive control, doxorubicin, has an $IC_{50}$ value of 1060 ± 43 nM against Hep G2 cancer cells. In comparison, compounds **2**, **3**, and **4** were more potent than the positive control. Compound **2** exhibited an $IC_{50}$ value of 161.3 ± 41 nM, compound 3 had an $IC_{50}$ value of 438.3 ± 33 nM, and compound **4** showed an $IC_{50}$ value of 400.4 ± 56 nM.[57]

In addition to testing the synthesized compounds against Hep G2 cells, they were also evaluated for their inhibition activity against colon cancer (Caco-2) cells. Among the compounds tested, compound **1** and compound **4** demonstrated potent inhibition activity at a concentration of 0.2 $\mu$M, with percentage inhibitions of 96 and 98%, respectively.



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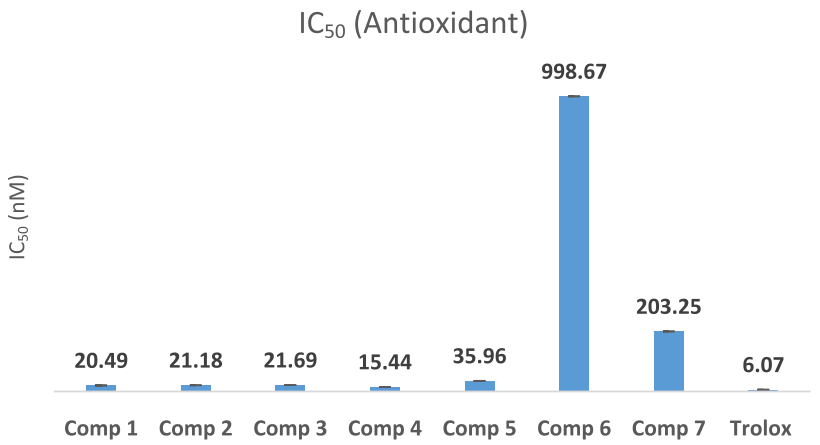


**Figure 4.** $IC_{50}$ of the antioxidant activities of the synthesized compounds.

Further analysis of the $IC_{50}$ values in Figure 3 reveals that compound **4** exhibited an $IC_{50}$ of 24.6 ± 8 nM against colon cancer cells, indicating its strong potency. However, compound **3** demonstrated even higher potency, with an $IC_{50}$ value of 9.6 ± 1.1 nM, making it the most potent compound among those tested. Comparing these $IC_{50}$ values to the positive control, doxorubicin, it can be observed that the synthesized compounds, especially compound **3** and compound **4**, exhibit higher potency. The $IC_{50}$ value of doxorubicin against colon cancer cells is 497 ± 0.36 nM, whereas compound **3** and compound **4** showed significantly lower $IC_{50}$ values, indicating their superior inhibitory activity against colon cancer cells.[58]

The overall results of cytotoxicity tests demonstrate the efficacy of the chemicals against the cancer cell type under study. However, it is critical to evaluate the active chemicals for their influence on normal cells while displaying low toxicity. Unfortunately, we were unable to undertake this assessment due to the lack of testing supplies during the study. This limitation serves as a valuable insight for future research, suggesting that such testing be incorporated into subsequent work.

**3.3. Antioxidant Activity.** The synthesized compounds were tested for their antioxidant activity using the DPPH assay. The results presented in Table 1 indicated variable antioxidant activity among the compounds, with most of them demonstrating moderate antioxidant activity. Compound **4** exhibited the highest inhibition percentage (39.4% ± 2.44) at a concentration of 50 μg/mL, indicating the strongest antioxidant activity among the synthesized compounds. Its $IC_{50}$ value was calculated to be 15.44 ± 6 nM. Compound **4** possesses a tertiary alcohol group, which could contribute to its increased antioxidant activity.

Compared with the synthesized compounds, it is noted that compounds with tertiary alcohol groups exhibited better antioxidant activity compared with compounds containing alcoholic groups coupled with cysteine. The $IC_{50}$ values for the synthesized compounds ranged from 15.44 to 998.67 nM, indicating their respective potency as antioxidants (Figure 4). The results were compared to the positive control, Trolox, which had an $IC_{50}$ value of 6.07 ± 0.8 nM; the antioxidant inhibition of compound **4** is nearly half that of Trolox, highlighting its strong antioxidant activity.[59]

**3.4. Cyclooxygenase Inhibition Activity.** The synthesized drugs were tested for their inhibition activity against cyclooxygenase isoenzymes COX-1 and COX-2. The results indicated good COX-2 inhibition activity for most of the synthesized compounds, with $IC_{50}$ values ranging from 4.37 to 129.9 nM. Compound **1** demonstrated the best $IC_{50}$ for COX-1 inhibition, with a value of 5.59 ± 0.51 nM. Additionally, compound **1** was the most effective inhibitor for COX-2, with an $IC_{50}$ of 4.90 ± 0.58 nM. Compound **7** also showed significant inhibition activity for COX-2, with an $IC_{50}$ of 4.37 ± 0.78 nM, and it exhibited good selectivity for COX-2 (selectivity value of 3.78). The results suggest that adding a cysteine group to the aromatic tertiary alcohol structure noticeably increases the COX-2 inhibition activity. Detailed $IC_{50}$ results can be found in Table 2, and the selectivity values for COX-2 are illustrated in Figure 5. The results indicate that almost all of the synthesized compounds were more potent than celecoxib, which is a common positive control with an $IC_{50}$ value of 40 nM. However, the positive control exhibited better selectivity (selectivity value of 8.5) toward COX-2 compared to the synthesized compounds.[60]

**Table 2. COX Inhibition Activity for the Synthesized Compounds**

| compounds | COX-1 $IC_{50}$ (nM) | COX-2 $IC_{50}$ (nM) | COX-2 selectivity |
|---|---|---|---|
| comp (**1**) | 5.59 ± 0.51 | 4.90 ± 0.58 | 1.14 |
| comp (**2**) | 5.63 ± 0.74 | 7.20 ± 0.58 | 0.78 |
| comp (**3**) | 12.79 ± 0.63 | 7.69 ± 0.55 | 1.67 |
| comp (**4**) | 6.52 ± 0.85 | 13.96 ± 1.70 | 0.44 |
| comp (**5**) | 6.02 ± 0.50 | 8.58 ± 0.56 | 0.70 |
| comp (**6**) | 42.40 ± 0.58 | 129.87 ± 1.71 | 0.33 |
| comp (**7**) | 16.72 ± 0.84 | 4.37 ± 0.78 | 3.78 |
| celecoxib (positive control) | 340.82 ± 23 | 40.04 ± 0.87 | 8.50 |

## 4. CONCLUSIONS

In this study, a group of tertiary alcohols of benzyl and phenyl xanthene and thioxanthene were successfully synthesized. Compound **4** showed the highest inhibition percentage, indicating the strongest antioxidant activity among the synthesized compounds. The synthesized compounds were also tested against various cancer cell lines, revealing that some of the compounds, such as compounds **2** and **4**, exhibited significant activity. This suggests the potential of these compounds as anticancer agents. Furthermore, the synthesized compounds were evaluated for their COX inhibition activities.


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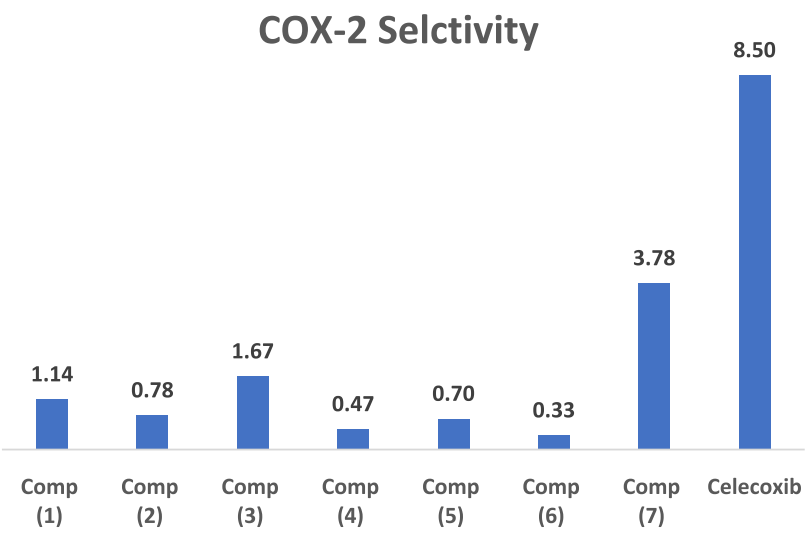


**Figure 5.** COX-2 inhibition selectivity of the synthesized compounds.

Compound 7 demonstrated good inhibition activity against COX-2 and displayed selectivity toward this enzyme. This finding indicates the compound's potential as an anti-inflammatory agent. Based on the promising results obtained, future work in this area could involve synthesizing analogues of xanthene and thioxanthene chemical core groups. These analogues can be designed to have structural variations to explore their impact on bioactivity. Subsequently, these newly synthesized compounds can be subjected to various bioactivity tests, with a specific focus on anticancer and anti-inflammatory activities.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

All data generated or analyzed during this study are included in this published article

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05695.

IUPAC names; chemical structures; and NMR spectra of all compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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