MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2025.20.2.10089

Synthesis, Molecular Docking, and In Vitro Activity Test of Thioxanthenol and Nitrothioxanthone Derivatives As Anticancer Agents

Putri Dian Anggraeni[1], Jumina Jumina[1]*, Chairil Anwar[1], Yehezkiel Steven Kurniawan[1]

[1]Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

*Corresponding author email: jumina@ugm.ac.id

Received December 18, 2023; **Accepted** July 09, 2025; **Available online** July 20, 2025

ABSTRACT. This research aimed to compare, synthesise, study molecular docking, and test the anticancer activity of thioxanthenol, 1-hydroxythioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone compounds through in silico and in vitro assays, highlighting their selective cytotoxicity and potential as novel anticancer scaffolds. These four compounds were obtained through reduction and nitration reactions of the thioxanthone. Thioxanthenol compound was obtained through the reduction of thioxanthone using sodium borohydride. The 1-hydroxythioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone compounds were obtained from the nitration of thioxanthone compounds. The compounds were characterised using FTIR, GC-MS, ^{1}H-NMR, and ^{13}C-NMR. In vitro cytotoxicity tests were performed using microtetrazolium (MTT) assays against T47D, WiDr, and Hela cancer cell lines and the Vero cell line as normal cells. The molecular docking process was studied to determine the in silico activity of the compounds with protein targets. The reduction reaction produced the thioxanthenol compound as a yellowish-white solid in 40.63% yield. The nitration reaction produced 1-hydroxythioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone compounds as light-yellow solids in 33.54%; 29.27%; and 31.71% yield, respectively. The synthesized compounds demonstrated selective anticancer activity against certain cancer cells. Thioxanthenol compound showed an IC_{50} value of 17.46 μg mL^{-1} on the WiDr cell line and nitrotioxanthone compound showed an IC_{50} value of 6.05 μg mL^{-1} on the T47D cell line. Molecular docking showed that the thioxanthone derivatives might act as the anticancer agent through inhibition of epidermal growth factor receptor (EGFR), P-glycoprotein, and Erα functions.

Keywords: anticancer, nitrothioxanthone, thioxanthenol, thioxanthone

INTRODUCTION

Pharmacology and chemistry scientists are constantly researching potential pharmaceutical compounds to provide new drugs against diseases with lower toxicity effects. According to GLOBOCAN 2022 data, over 19.9 million new cancer cases and nearly 9.7 million cancer deaths were reported worldwide. The most common cancers include breast, lung, and colorectal cancers, highlighting the urgent need for effective and selective anticancer agents (Bray et al., 2024). However, anticancer drugs often become resistant and have low selectivity, destroying normal cells alongside cancer cells. Thioxanthone compounds are well-considered anticancer agents due to their bioactivity and easy substitution, especially hydrogen abstraction (Zhao et al., 2020). Thioxanthenol is synthesized from thioxanthone using sodium borohydride or lithium aluminium hydride reagents. Reduction reactions have been carried out using thioxanthone sulfoxide and sodium borohydride catalysts (Ternay and Chasar, 1967). Amanatie et al. (2017) nitrated xanthone using nitric acid, producing a yellowish-brown solid of 2-nitroxanthone.

On the other hand, hydroxyxanthone, a potent anticancer agent with a small IC_{50} value, has been shown to be effective in treating MCF-7, WiDr, and HeLa cell lines (Fatmasari et al., 2022). Chlorothioxanthones like 4-chloro-3-(4-chlorophenylthio)-9*H*-thioxanthen-9-one and 3-(4-bromophenylthio)-4-chloro-9*H*-thioxanthen-9-one provide good inhibition to cancer cells (Chen et al., 2019). These compounds inhibit P-glycoprotein through the 4-alkoxyl or 4-hydroxyl group and amino to the C-1 position. They can also inhibit polymer inclusion membrane (PIM1) proteins through hydrophobic interactions with Ile104, Leu120, Val126, Leu174, and Ile185 amino acid residues similar to compound HM107-g (Palmeira et al.,2012; Brikci-Nigassa et al., 2020).

Thioxanthone derivatives can also inhibit Platelet-derived growth factor receptors (PDGFR) and epidermal growth factor receptor (EGFR) proteins. It was reported that 4-iodo-1,3-dihydroxy-thioxanthone has the strongest binding energy to Cys673 and Met769 amino acid residues in PDGFR and EGFR, respectively. The 4-iodo-1,3-dihydroxy-thioxanthone

can also perform hydrophobic interactions at Phe723, Val726, Ala743, Leu844, and Thr854 amino acid residues of EGFR. Inhibition against xanthotoxol is also achieved through hydrophobic interactions and polar hydrogen interactions (Acharya et al., 2019; Hermawan et al., 2020). The research aimed to synthesize thioxanthenol and nitrothioxanthone as the anticancer compounds. These compounds were obtained from the reduction and nitration reactions of thioxanthone. These compounds were tested for anticancer activity using the MTT assay method against various cancer cells, and then in silico investigation was performed to understand their anticancer mechanism.

EXPERIMENTAL SECTION

Materials and Instruments

The materials used in this study were 97% thioxantone, ethanol, glacial acetic acid, nitric acid, sulfuric acid, tetrahydrofuran, dichloromethane, diethyl ether, anhydrous sodium sulfate (Merck), and sodium borohydride (Loba Chemie). The materials used for the anticancer test included T47D breast cancer cells, HeLa cervical cancer cells, WiDr colon cancer cells, Vero normal cells, Dulbecco's Modified Eagle Medium (DMEM), Medium 199, Fetal Bovine Serum (FBS), Phosphate Buffer Solution (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pen strep, fungizone, doxorubicin, cisplatin, 5-flourouracil, trypsin enzyme, and sodium dodecyl sulfate (SDS). This study used various equipment for synthesized products, including a rotary evaporator Buchi type R-114, an Electrothermal melting point device 9100, Shimadzu Prestige-21 for infrared spectra, JEOL JNMECA 500 MHz for NMR spectra, and GC-MS QP2010S Shimadzu for GC-MS analysis. The anticancer assay equipment included a Sartorius mass balance, Hirayama autoclave, Labconco airflow, conical tube, Heraeus 5% CO_2 incubator, DIVAC vacuum filter, counting device, Axiovert 25 inverted microscope, Thermolyne vortex, Eppendorf tube, Sorvall centrifuge, and BIO-RAD Benchmark ELISA reader.

Synthesis of Thioxanthenol

Thioxanthone (0.32 g, 1.51 mmol) was dissolved in 50 mL of ethanol:tetrahydrofuran (4:1) and stirred for 15 minutes. The solution was then added to sodium borohydride and refluxed for 2 hours. TLC was used to monitor the reaction progress. Then, distilled water was added to precipitate the product and the solid was filtered and dried. The FTIR, NMR, and GC-MS instruments were used to characterize the product.

Synthesis of Nitrothioxanthone

Thioxanthone of (2.45 g, 11.54 mmol) was dissolved in glacial acetic acid and stirred at 0 °C for 10 minutes. Nitric acid (1.0 mL) and sulfuric acid (1.3 mL) were added, and the mixture was stirred for 8 hours. The reaction was monitored using TLC. The resulting yellow solid was filtered, washed, and dried. The solvent was removed by evaporation, and the solid was recrystallized from methanol. The product was characterized using FTIR, NMR, and GC-MS instruments.

Anticancer Activity Test Against Cancer Cells

Cytotoxicity test on cancer cells was carried out by cell proliferation method. The cell lines were thawed, added with trypsin, and counted. Cell line (100 μL) was placed into each well on a 96-well plate and incubated for 24 hours. Sample (5 mg) was dissolved in DMSO (100 μL) and then samples were diluted in a culture media with a series concentration of 7.81, 15.62, 31.25, 62.5, 125, 250, and 500 g mL^{-1}. Aliquots (100 μL) of each concentration were added to each well. The cell line was incubated again for 24 hours. The culture medium was removed and cleaned with PBS. The MTT solution (100 μL) was added to a 96-well plate and incubated for 4 hours. A stopper solution (100 μL) was then added to each well. The plate was incubated overnight at room temperature. The absorbance of the solution was recorded using an ELISA reader at a wavelength of 595 nm to calculate IC_{50} values.

Molecular Docking

The molecular docking process begins by downloading the 3D structure of a protein from the Protein Databank website. The proteins used as the target in this study are epidermal growth factor receptor (EGFR) protein tyrosine kinase with PDB ID of 1M17 (Stamos el al., 2002), P-glycoprotein with PDB ID of 2HYD (Dawson and Locher, 2006), and estrogen receptor alpha (ERα) with PDB ID of 3ERT (Shiau et al., 1998). Preprepared protein targets and native ligands are processed through Autodock Vina software (Trott & Olson, 2010), and the grid position is determined to obtain a ligand conformation with a Root Mean Square Deviation (RMSD) value less than 2 Å. If these conditions are met, the molecular docking process between the target protein and the synthesized compound can be carried out. The used grid box size is 20×20×20 Å. The structure of the synthesized thioxanthone derived compound is optimized before molecular docking with the protein target using Gaussian 5.0 software.

RESULT AND DISCUSSION

Synthesis of Thioxanthenol Through Reduction Reaction

Thioxanthone compounds have the same structure as xanthone compounds, but with a sulfur atom in their cyclic ring. Both compounds have carbonyl groups that can be reduced to hydroxyl group (Ternay and Chasar, 1967). The reduction reaction occurs by attacking the carbonyl group on the thioxanthone cyclic ring, forming a C–H bond and a C–O (alkoxy anion), and breaking this carbonyl bond to produce an alcohol. This study used a $NaBH_4$ reagent and a combination of solvents with an ethanol ratio of 4:1. The synthesis produced thioxanthenol in 40.63% yield

as a light yellow solid with a melting point of 98.50 °C. The melting point of the synthesized thioxanthenol in this work is in accordance with the reference literature, which is 97.40-105 °C (Chemicalbook, 2016). Overall synthetic route is illustrated in **Scheme 1**. Thioxanthenol was characterized using FTIR, GC-MS, ^{1}H-NMR, and ^{13}C-NMR spectrometers. The FTIR spectra (**Figure 1**) show a shrinking carbonyl peak at 3310 cm^{-1}, indicating a successful reduction of the carbonyl group to a hydroxyl group.

The GC-MS characterization revealed the formation of thioxanthenol compound as a dominant peak at 25.70 minutes with relative abundance of 63.84% and molecular ion fragment of 214. Thioxanthenol produced a fragmentation pattern with abundant fragments at m/z = 197. Two other peaks, thioxanthene and thioxanthone were also found at retention times of 22.06 and 28.42 minutes with percentages of 23.93% and 12.23%, respectively. These compounds formed due to unstable thioxanthenol, causing it to dissociate into thioxanthene and thioxanthone. This also occurred in the xanthydrol compound which was disproportionated into xanthone and xanthene (Shi et al., 2021).

The thioxanthenol compound was characterized using ^{1}H-NMR and ^{13}C-NMR in DMSO-d6 solvent. The ^{1}H-NMR spectrum (**Figure 2**) revealed five types of aromatic protons, with protons 1 and 8 appearing as doublets, protons 2 and 7 appearing as triplets, protons 3 and 6 appearing as triplets, and protons 4 and 5 appearing as doublets. The C–OH proton was found as a doublets at 6.37 ppm. The ^{13}C-NMR spectrum (**Figure 3**) revealed six carbon atoms in the aromatic ring and one carbon atom binding to hydroxyl (–OH).

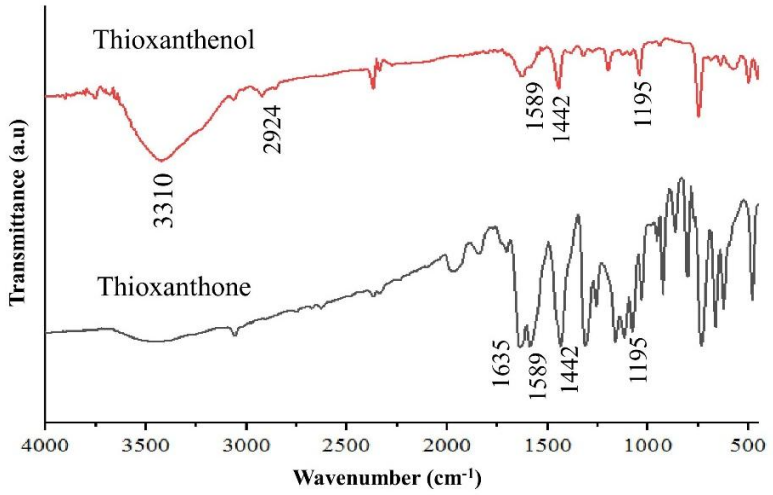

Figure 1. The 1H-NMR spectrum of thioxanthenol compound

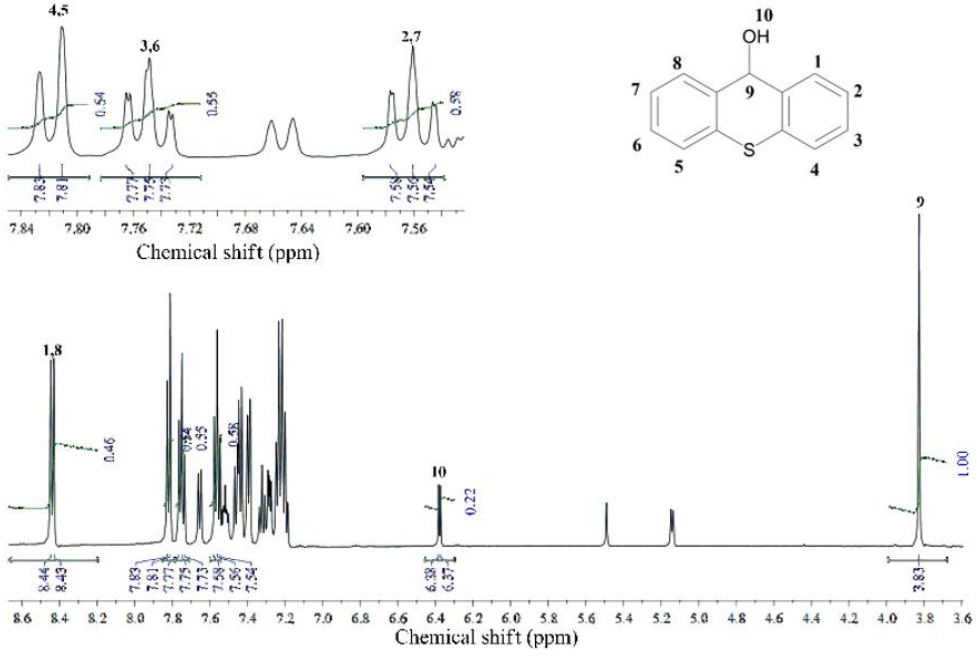

Figure 2. The ^{13}C-NMR spectrum of thoxanthenol compound

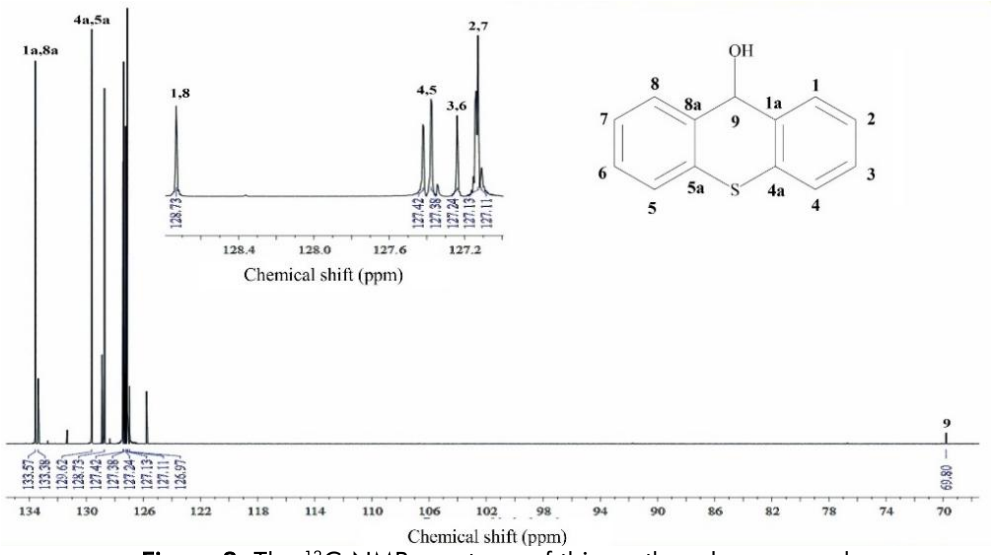

Figure 3. The ^{13}C-NMR spectrum of thioxanthenol compound

Synthesis of Nitrothioxanthone Through Nitration Reaction

The thioxanthone was nitrated to produce 2-nitrothioxanthone due to its aromatic ring at para and ortho positions. Amanatie et al. (2017) has been successfully synthesized 2-nitroxanthone using nitric acid in glacial acetic acid. In this work, the nitrothioxanthone compound was obtained in the form of a mixture of light-yellow solids in 66.99% yield with a melting point of 276 °C. GC-MS characterization reveals three products, i.e., 1-hydroxythioxanthone (33.63%), 4-nitrothioxanthone (29.46%), and 2-nitrothioxanthone (31.79%). This synthetic pathway is outlined in **Scheme 2**. The mass spectrum of 1-hydroxythioxanthone was identified at m/z = 228, while the nitroxanthone products were detected at 257 m/z. The fragmentation pattern of each compound is shown in **Figure 4**.

The formation of 1-hydroxythioxanthone product in nitration reactions occurs when positive species NO_2^+ binds with the π-aromatic system through oxygen or H^+ cation. The double bond stabilizes the intermediate compound, and partially positively charged carbon atoms interact with partially negatively charged oxygen atoms to form hydroxy substituents. On the other hand, both 4-nitrotioxanthone and 2-nitrothioxanthone compounds are directly formed by the reaction between NO_2^+ agent and the aromatic ring. The FTIR spectrometer (**Figure 5**) reveals hydroxy group at 3441 cm^{-1}, indicating the 1-hydroxythioxanthone compound. Nitro absorption signals at 1574 and 1342 cm^{-1}, correspond to the presence of 4-nitrothioxanthone and 2-nitrothioxanthone. The other absorption peaks at 1442, 1589, 2924, and 1172 cm^{-1} represent aromatic C=C, C_{sp2}-H, and C–S–C bonds, respectively.

The structures of nitrothioxanthone compounds were also analyzed using ^{1}H-NMR and ^{13}C-NMR spectrometers in DMSO-d6 solvent. The ^{1}H-NMR spectra revealed 12 types of protons from 1-hydroxyithioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone compounds as shown in **Figure 6**. Meanwhile, the ^{13}C-NMR spectrum (**Figure 7**) reveals 12 aromatic carbon atoms of 1-hydroxythioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone with 3 carbon atoms binding to hydroxy and nitro groups.

Anticancer Activity Assay of Thioxanthenol and Nitrothioxanthone Compounds

The American National Cancer Institute (NCI) determine the effectiveness of a compound's inhibition activity based on the IC_{50} value obtained during the MTT assay method. The category of the effectiveness of anticancer agents is as follows: a very active inhibitor (IC50 < 20 μg mL-1), a moderate inhibitor (IC50 is between 21–200 μg mL-1), and an inactive inhibitor (IC50 is between 201–500 μg mL-1).

All synthesized compounds were purified via recrystallization prior to biological testing, and their structures were confirmed through FTIR, NMR, and GC-MS analyses. The study conducts an anticancer assay on various cancer cells (T47D, HeLa, and WiDr) and Vero normal cells and the results are listed in **Table 1**. Results showed that thioxanthenol was highly active in inhibiting WiDr cancer cells and was moderately active against T47D cancer cells. Meanwhile, nitrothioxanthone compounds were highly active in inhibiting T47D cancer cells. On the other hand, WiDr cancer cells are very well inhibited by thioxanthone compounds with IC_{50} values smaller than the 5-fluorouracil. Thioxanthone also showed moderate inhibition to HeLa cells but was inactive against T47D cancer cells.

The selectivity index measures a compound's selectivity, demonstrating its effectiveness as the anticancer agent. A score below 3 indicates non-selectivity, while a score above or equal to 3 indicates high selectivity (Sirait et al., 2019). Thioxanthone, thioxanthenol, and nitrothioxanthone compounds exhibit a high selectivity index (SI≥3) on T47D, HeLa, and/or WiDr cancer cells as shown in **Table 2**.

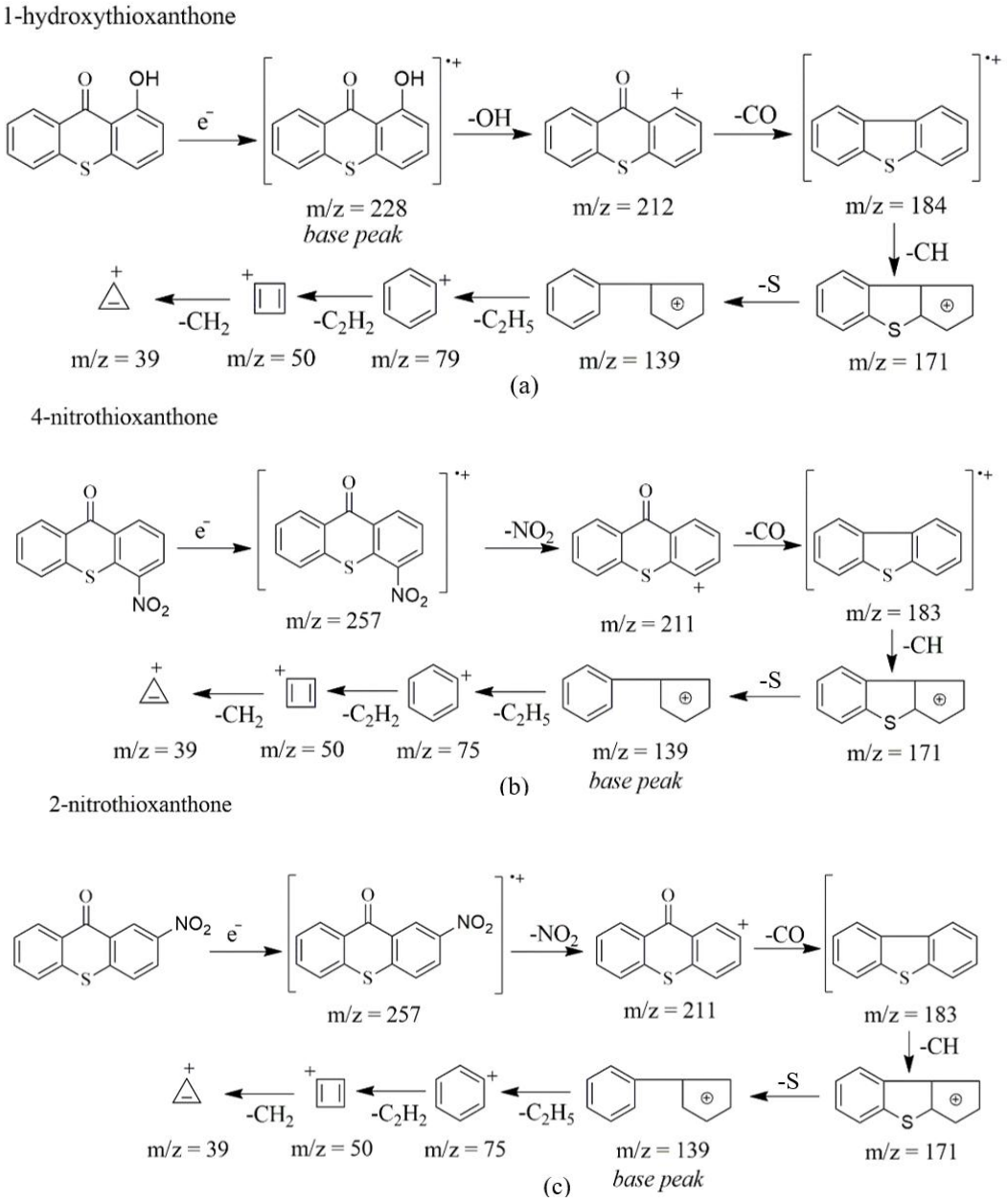

Figure 4. Possible fragmentation pattern of (**a**) 1-hydroxythioxanthone, (**b**) 4-nitrothioxanthone, and (**c**) 2-nitrothioxanthone

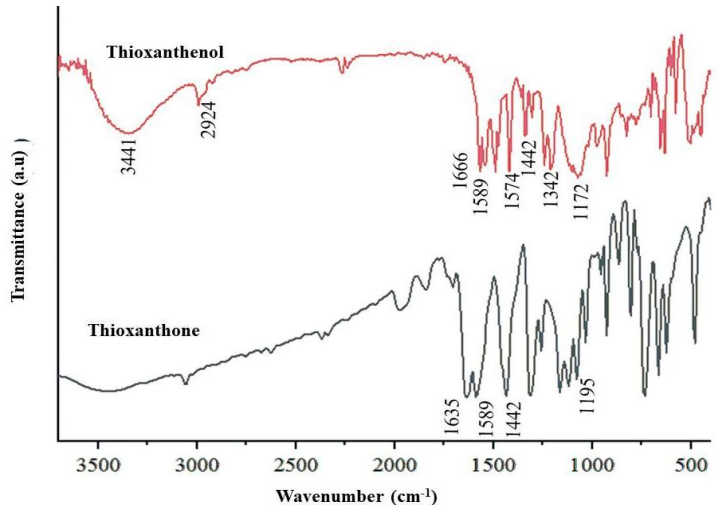

Figure 5. The FTIR spectra of nitrothioxanthone (red line) and thioxanthone (black line) compounds

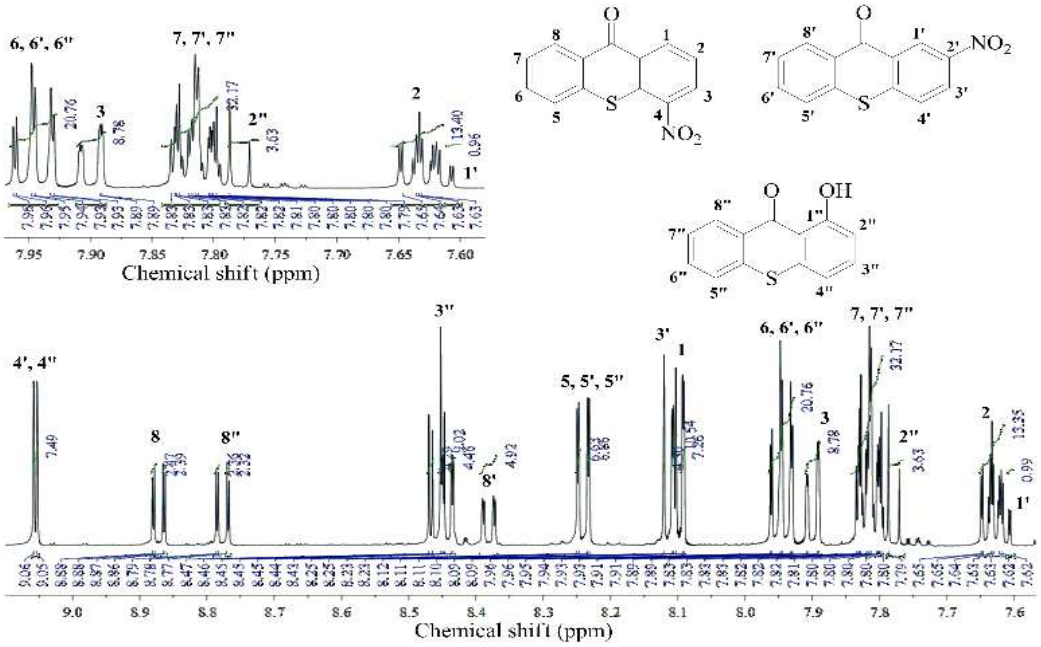

Figure 6. The ^{1}H-NMR spectrum of nitrated thioxanthone

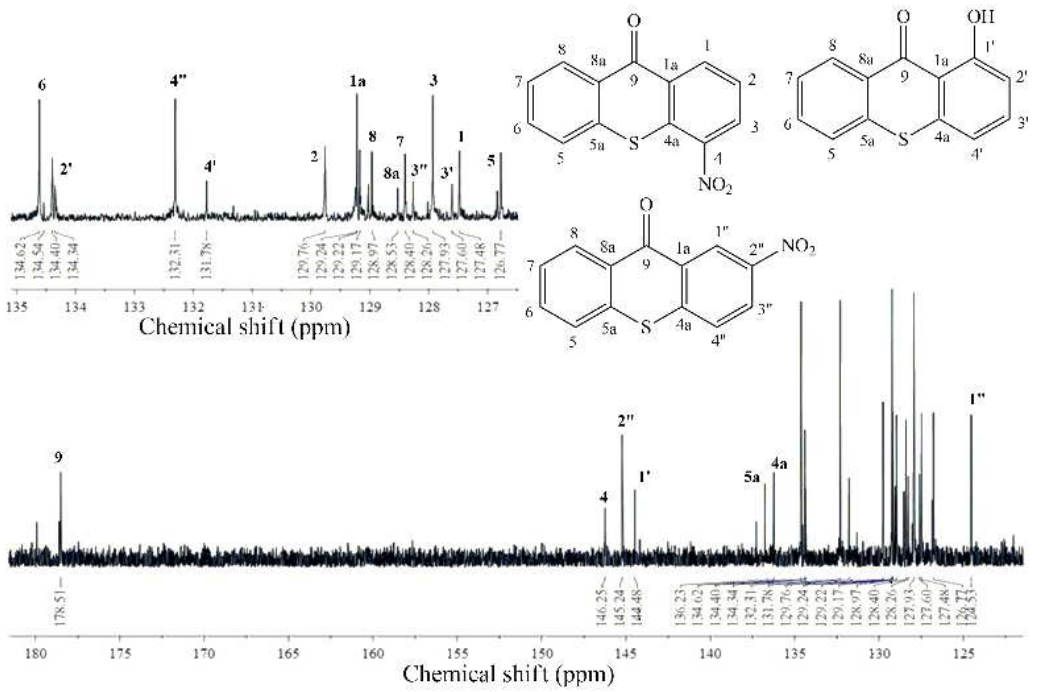

Figure 7. The ^{13}C-NMR spectrum of nitrated thioxanthone

Table 1. Anticancer activity assay result

Compounds	IC_{50} (μg mL^{-1})			
	T47D	HeLa	WiDr	Vero
Thioxanthone	6496.36	158.85	5.74	969.66
Thioxanthenol	70.88	2441.00	17.46	234.50
Nitrothioxanthone	6.05	41412.30	402.55	142888.00
Doxorubicin	0.21	-	-	115.42
Cisplatin	-	68.80	-	-
5-Fluorouracil	-	-	36.89	-

Table 2. The selectivity index of the test compounds

Compounds	Selectivity index		
	T47D	HeLa	WiDr
Thioxanthone	0.11	6.10	168.96
Thioxanthenol	3.31	0.10	13.43
Nitrothioxanthone	23637.39	3.45	354.95

These findings indicate that the synthesized compounds possess selective cytotoxic activity depending on the cancer cell type, suggesting differences in cellular sensitivity or compound uptake. The relatively low IC_{50} values in certain cell lines reflect strong antiproliferative potential, especially for WiDr and T47D. Such selectivity is important in anticancer drug development as it may reduce toxicity to non-target tissues. However, to fully understand the therapeutic potential of these compounds, further studies are required to elucidate the specific cellular mechanisms involved in their I nhibitory effects. As a preliminary study, we employed molecular docking process to observe whether these synthesized compounds against three well-known targeted protein receptors for cancer therapies, i.e., EGFR, P-glycoprotein, and ERα. EGFR regulates cancer cells' growth, as well as their proliferation, differentiation, and migration while P-glycoprotein and ERα are crucial for drug efficacy and DNA activation in cancer cells, respectively.

Molecular Docking of Thioxanthenol and Nitrothioxanthone Compounds

Molecular docking is a method used to predict the binding affinity and bioactivity of a compound *in silico*. It involves two stages: redocking and docking. Redocking is used to establish a valid docking method based on ligand conformation and RMSD value. Docking with a proposed compound involves the compound acting as a ligand with a protein separated from its native ligand. A smaller binding affinity value indicates a stronger interaction (Astalakshmi et al., 2022). The thioxanthone derivatives in this work were geometry-optimized using Gaussian 5.0 software. The density functional theory (DFT) and semi-empirical methods were used for optimization, as they don't significantly affect the bonding of the compound with its target molecule. Therefore, this study used the PM3 semiempirical method for the optimization of the proposed compounds, i.e., thioxanthenol, 2-nitrothioxanthone, 4-nitrothioxanthone, and 1-hydroxythioxanthone.

The PM3 semiempirical method was selected due to its superior performance in previous studies compared to PM6 (Male et al., 2018). In this study, PM3 was used solely for initial geometry optimization to provide a reasonable starting structure for molecular docking. However, it is important to note that AutoDock Vina applies its own internal force field and conformational sampling, and therefore does not strictly rely on the starting geometry during the docking process. PM3 is faster and more accurate than *ab initio* and AM1. It is particularly effective for compound structures with nitrogen atoms, such as –NH_2, –NHR, and –NR_2 in quinoacridinium compounds (Hadanu, 2019). The PM3 semi-empirical method optimizes the proposed compounds' structures before docking them with target receptors, i.e., EGFR, P-glycoprotein, and ERα proteins. It involves understanding the formed interactions through hydrogen, hydrophobic, and van der Waals bonds. Hydrogen bonds are stronger and more stable, while hydrophobic bonds are formed between nonpolar molecules that cannot form hydrogen bonds with water molecules. While, van der Waals bonds are weaker than hydrophobic bonds (Luanphaisarnnont, 2009).

EGFR Protein

A compound has been evaluated as the EGFR inhibitor, which EGFR regulates cancer cells' growth, as well as their proliferation, differentiation, and migration. A molecular docking study was conducted to determine the interaction between the compound and the EGFR protein. The native ligand (erlotinib) obtained a redocking pose similar to the previous one with RMSD value of 1.54 Å so that this redocking method can be used to dock the proposed compound to EGFR protein. The erlotinib ligand forms a hydrogen bond with the Gln767 amino acid residue. This result is in accordance with the results of previous research conducted by Hermawan et al. in 2019. Hydrogen bonds were formed at the redocking stage, with weak hydrogen bonds formed by the interaction of the oxygen atoms of Leu694, Gly772, and Gln767 with the erlotinib compound. Additional interactions occurred in hydrophobic and van der Waals bonds.

The thioxanthenol compound (**Figure 8a** and **Figure 8e)** forms strong hydrogen bonds with the EGFR's amino acid residues, demonstrating its strong inhibitory activity. The formed interactions also involve hydrogen bonds with Asp381 and Thr830. The van der Waals occurs at the main amino acid residues, i.e., Glu738, Met742, and Thr766, which also bind to the native ligand erlotinib. In molecular docking, the more interactions that occur, the stronger the binding energy (Ferreira et al., 2015). The binding affinity value between the compound and the protein is slightly higher than the native ligand which is -7.40 kcal mol^{-1}, possibly due to the different hydrogen bonds formed.

This study also analyzed the molecular docking of nitrated compounds of thioxanthone, resulting in 1-hydroxythioxanthone, 2-nitrothioxanthone, and 4-nitrothioxanthone, to determine their effectiveness in inhibiting EGFR. The compounds interact through van der Waals and hydrophobic bonds, with no hydrogen bond interaction between the compounds and proteins. The 1-hydroxythioxanthone (**Figure 8b** dan **Figure 8f**) formed new bonds with Leu694, Met769, and Asp831, while the 4-nitrotioxanthone (**Figure 8c** dan **Figure 8g)** generated new bonds with Met769 and Leu694 amino acids. The 2-nitrothioxanthone (**Figure 8d** dan **Figure 8h)** formed new bonds with Met769, Leu764, and Glu767 amino acids. Hydrophobic bonds form with thioxanthone-derived compounds with nitro substitutions have smaller binding affinity and energy production, resulting in more stable ligand bonds and increased activity with protein (Fu et al., 2018; Murthy and Bala Narsaiah, 2019). The 2-nitrothioxanthone compound has a smaller binding affinity of -8.00 kcal mol^{-1}, indicating a stronger bond between the protein and the compound. This differs from the hydrogen bond found in Hermawan et al. (2019) research. The docking data shows that 2-nitrothioxanthone compounds inhibit EGFR, blocking tyrosine-kinase from binding to the receptor, making it inactive for cancer cell growth.

P-glycoprotein

P-glycoprotein, a crucial protein in cell entry and exit, is crucial for drug efficacy in cancer cells. Proposed compounds inhibit P-glycoprotein proteins through hydrogen bonds, hydrophobic bonds, and van der Waals bonds. Hydrophobic bonds interact with Leu478, and van der Waals bonds form interactions with Met375, Val532, and Gly480. The target protein docked with a thioxanthenol compound, forming hydrophobic and van der Waals bonds.

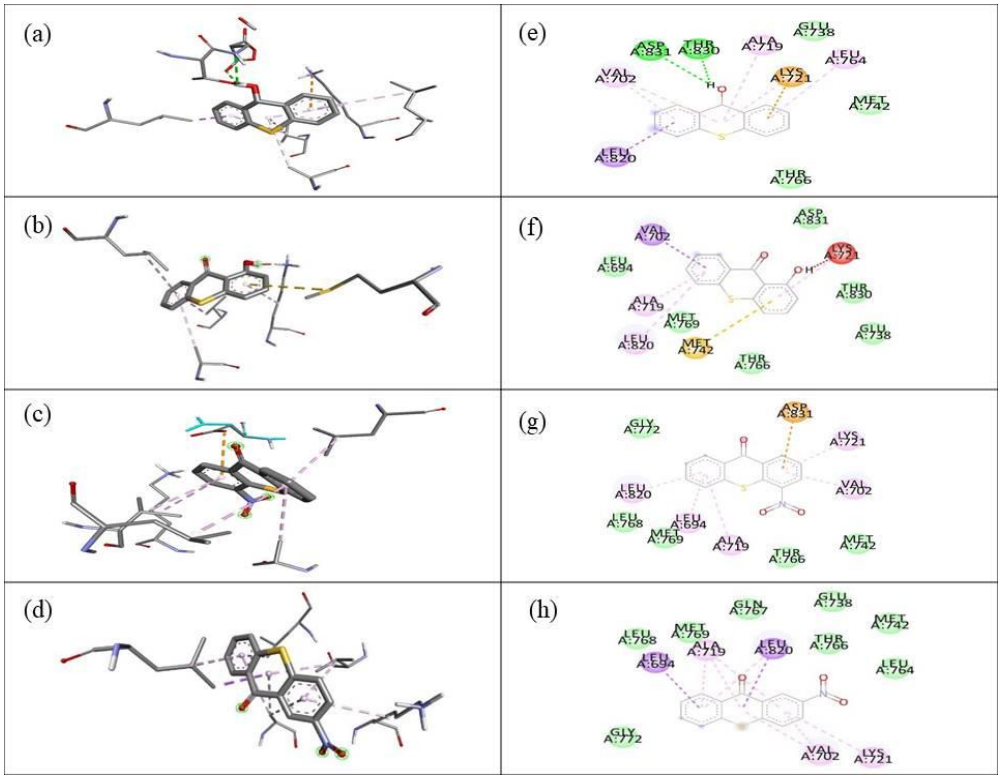

Figure 8. Interaction of each compound with EFGR protein: (**a**) thioxanthenol in 3D, (**b**) 1-hydroxythioxanthone in 3D, (**c**) 4-nitrothioxanthone in 3D, (**d**) 2-nitrothioxanthone in 3D, (**e**) thioxanthenol in 2D (**f**) 1-hydroxythioxanthone in 2D, (**g**) 4-nitrothioxanthone in 2D, and (**h**) 2-nitrothioxanthone in 2D.

Table 4. Binding affinity values of tested compounds and native ligand against EGFR

Compounds	Binding affinity (kcal mol^{-1})
Native ligand	-7.40
Thioxanthenol	-7.30
1-hydroxythioxanthone	-7.30
2-nitrothioxanthone	-7.80
4-nitrothioxanthone	-8.00

Hydrophobic bonds are stronger than van der Waals bonds, with additional interactions with Pro464. The binding affinity is -8.00 kcal mol^{-1}, but higher than the native ligand binding affinity of -9.30 kcal mol^{-1}. This indicates that the thioxanthenol compound's hydrophobic and van der Waals bonds do not provide a stronger bond than the native ligand. Thioxanthenol (**Figure 9a** and **Figure 9e)** interacted through hydrogen, hydrophobic, and van der Waals bonds. The hydrogen bond indicates a strong bond between the compound and the target protein. However, the hydrogen-bonded amino acid residue differs from the native ligand's amino acid residue (Wang et al., 2015; Kumar et al., 2016). A compound's pharmacological effect is more like its standard ligand if it interacts with its major amino acid residue. Hydrophobic bonds form new interactions with Ala113 and Pro464, while van der Waals bonds form new interactions with Asn350, Thr469, and Leu463. Unfortunately, the binding affinity of 1-hydroxythioxanthone compounds (**Figure 9b** and **Figure 9f**) is still greater than the native ligand.

The 2-nitrothioxanthone and 4-nitrothioxanthone compounds interact through van der Waals and hydrophobic bonds with native ligands. The 2-nitrothioxanthone forms (**Figure 9d** and **Figure 9h**) new interactions with Ala113 and Pro464, while 4-nitrothioxanthone (**Figure 9c** and **Figure 9g**) has the largest number of interactions, resulting in similar pharmacological effects to its standard ligand. The binding affinity is -9.00 kcal mol^{-1}, which closed to the native ligand's binding energy. The 4-nitrothioxanthone compound was found to be effective as an anticancer agent due to its smaller binding affinity and common interactions with amino acid residues in the ATP pocket of P-glycoprotein.

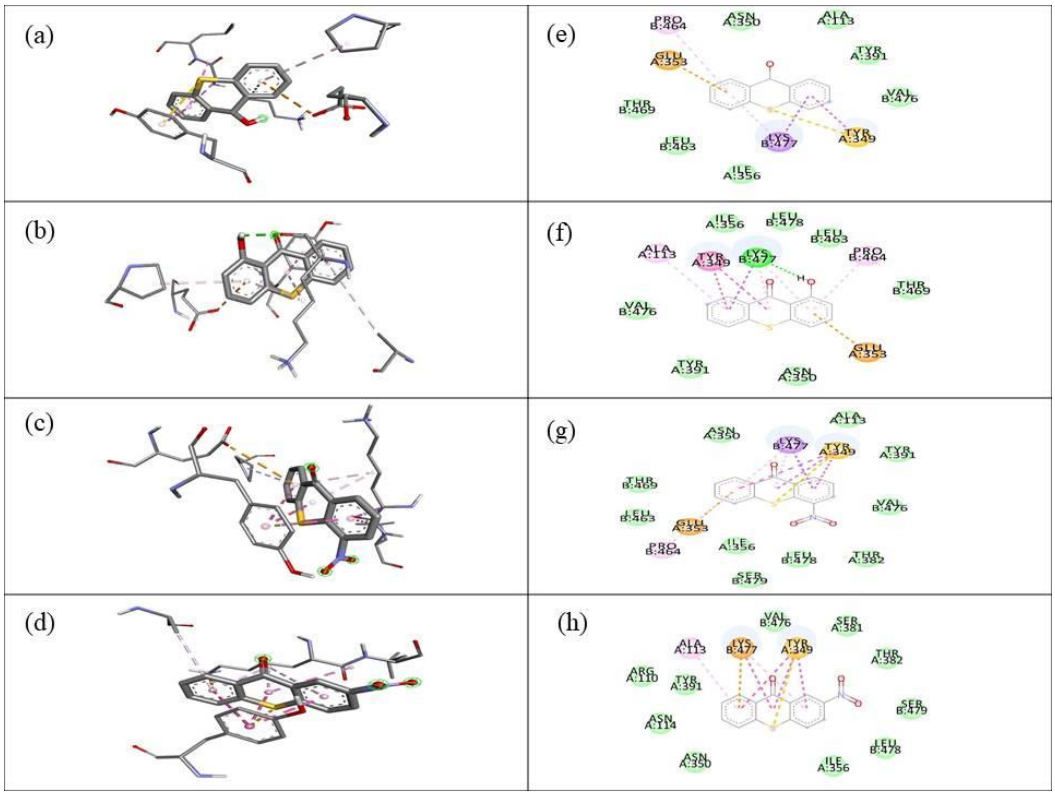

Figure 9. Interaction of each compound on P-glycoprotein: (**a**) thioxanthenol in 3D, (**b**) 1-hydroxythioxanthone in 3D, (**c**) 4-nitrothioxanthone in 3D, (**d**) 2-nitrothioxanthone in 3D, (**e**) thioxanthenol in 2D (**f**) 1-hydroxythioxanthone in 2D, and (**g**) 4-nitrothioxanthone in 2D, (**h**) 2-nitrothioxanthone in 2D.

Table 5. Binding affinity values of tested compounds and native ligand against P-glycoprotein

Compounds	Binding affinity (kcal mol^{-1})
Native ligand	-9.30
Thioxanthenol	-8.00
1-hydroxythioxanthone	-8.30
2-nitrothioxanthone	-9.00
4-nitrothioxanthone	-9.00

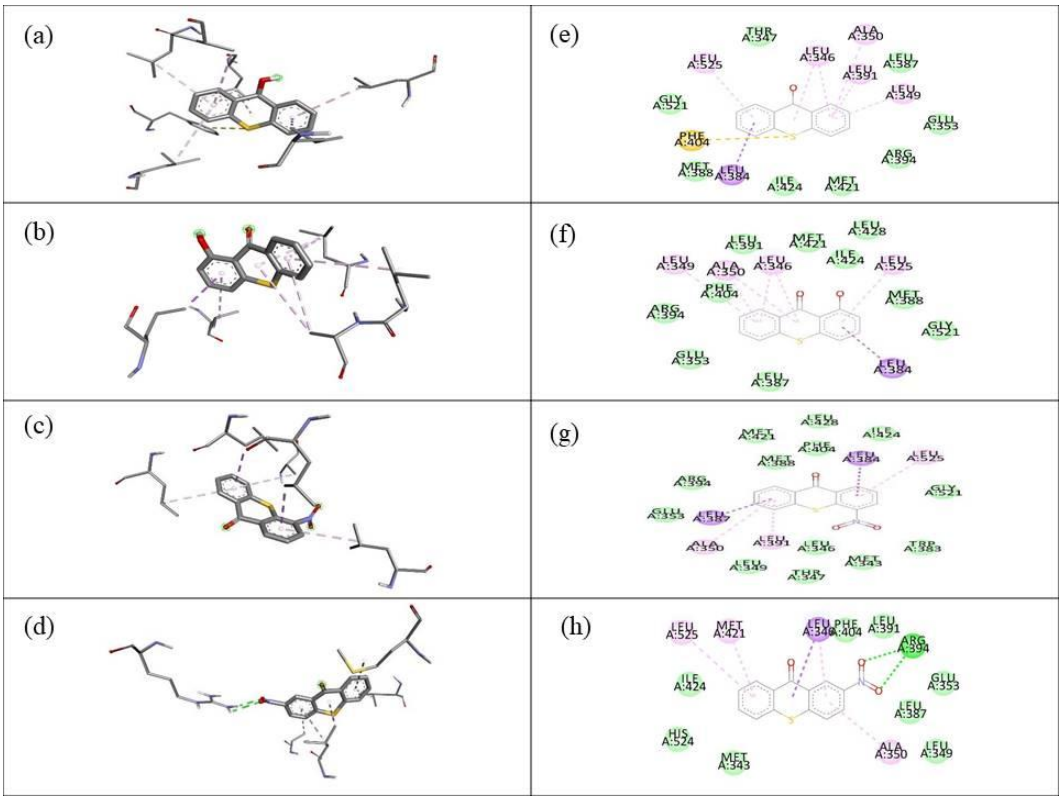

Figure 10. Interaction of each compound with ERα: (**a**) thioxanthenol in 3D, (**b**) 1-hydroxythioxanthone in 3D, (**c**) 4-nitrothioxanthone in 3D, (**d**) 2-nitrothioxanthone in 3D, (**e**) thioxanthenol in 2D (**f**) 1-hydroxythioxanthone in 2D, (g) 4-nitrothioxanthone in 2D, and (**h**) 2-nitrothioxanthone in 2D

Table 6. Binding affinity values of tested compounds and native ligand against ERα

Compounds	Binding affinity (kcal mol^{-1})
Native ligand	-9.90
Thioxanthenol	-7.80
1-hydroxythioxanthone	-7.80
2-nitrothioxanthone	-7.70
4-nitrothioxanthone	-8.00

ERα Protein

ERα activates DNA by binding to estrogen hormone, causing RNA formation, which can increase cancer cell numbers and activity. The thioxanthenol, 2-nitrothioxanthone, 4-nitrothioxanthone, and 1-hydroxythioxanthone have been studied through molecular docking against ERα. ERα protein was redocked with its native ligand, resulting in a molecular docking method with an RMSD value of 1.14 Å. Interactions occurred through hydrogen, hydrophobic, and van der Waals bonds The interacting amino acid residues are the same as the redocking results of Narko et al. in 2017. The thioxanthenol compound (**Figure 10a** and **Figure 10e**) forms hydrophobic and van der Waals bonds, resulting in greater binding affinity than the native ligand. However, the strength of hydrophobic and van der Waals bonds is not strong enough to inhibit estrogen binding to ERα.

The study reveals that 1-hydroxythioxanthone (**Figure 10b** and **Figure 10f**) interacts with the target protein through hydrophobic and van der Waals bonds, resulting in a binding affinity value of -7.80 kcal mol^{-1}. Hydrophobic bonds formed with the main amino acid residues, such as Ala350 and Leu525. The van der Waals bonding occurs with two main amino acid residues, namely Phe404 and Gly521. Nitrothioxanthone compounds interact with target protein amino acid residues through hydrophobic and van der Waals bonds. The 4-nitrotioxanthone (**Figure 10c** and **Figure 10g**) interacts through hydrophobic bonds and one new residue, Leu384, while the 2-nitrothioxanthone compound has a hydrogen bond, making ERα inhibition stronger. The 2-nitrothioxanthone compound (**Figure 10d** and **Figure 10h**) also binds through hydrophobic bonds at its main amino acid residues and additional van der Waals bonds at a new Arg394 amino acid residue.

Narko et al. (2017) and Acharya et al. (2019) performed docking of their proposed compounds and the same hydrogen bond occurred at Arg394 amino acid residue. The 2-nitrothioxanthone compound, with a smaller binding affinity (-8.00 kcal mol^{-1}), may inhibit ERα activation in cancer cells as its binding to ERα is more difficult to release, unlike other investigated compounds in this work.

It is important to note that molecular docking only provides predictive binding insights and does not fully reflect in vivo conditions. However, a significant limitation of the current study lies in the absence of in vivo testing. Although the compounds showed promising in vitro and in silico results, biological complexity in living organisms may influence pharmacokinetics, toxicity, and therapeutic efficacy differently. Without in vivo data, the full therapeutic potential and safety profile of these compounds cannot yet be conclusively determined.

Thioxanthenol derivatives exhibit several advantages that support their potential as anticancer candidates. These include their high selectivity towards certain cancer cell lines, ease of synthesis from readily available thioxanthone precursors, and favorable biological activity as observed in both in vitro cytotoxicity and molecular docking studies. The compounds also follow drug-likeness parameters such as Lipinski's Rule of Five and demonstrate acceptable ADMET profiles, further validating their pharmaceutical relevance.

Future research will focus on structural optimization of the synthesized derivatives to enhance their binding affinity and selectivity. In vivo animal studies will be conducted to evaluate pharmacodynamic and pharmacokinetic behaviors, as well as systemic toxicity. Furthermore, clinical validation through preclinical trials in collaboration with biological laboratories is planned to verify the efficacy and safety of the most promising candidates, ultimately supporting their development as viable anticancer drugs.

CONCLUSIONS

Thioxanthenol compound has been successfully synthesized from thioxanthone using sodium borohydride reagent with a yield of 40.63%. The nitration reaction of thioxanthone with nitric acid and sulfuric acid was successfully carried out to produce 1-hydroxylthioxanthone, 4-nitrothioxanthone, and 2-nitrothioxanthone in 33.54%, 29.27%, and 31.71% yield, respectively. The nitrothioxanthone compounds had a higher IC_{50} value for WiDr colon cancer cells than thioxanthenol compound, but they gave a smaller IC_{50} value for T47D breast cancer cells. The nitrated thioxanthones also selective for various cancers, especially for HeLa cervical cancer cells. The 2-nitrothioxanthone compound interacts with EGFR and P-glycoprotein through hydrophobic bonds and van der Waals, suggesting it could act as an anticancer agent for HeLa cancer due to its interactions with both receptors. To strengthen the therapeutic implications of these findings, future studies involving in vivo validation in collaboration with biological experiments are planned.

REFERENCES

Acharya, R., Chacko, S., Bose, P., Lapenna, A., & Pattanayak, S. P. (2019). Structure based multitargeted molecular docking analysis of selected furanocoumarins against breast cancer. *Nature, 9*(15743), 1-13. https://doi.org/10.1038/s41598-019-52162-0

Amanatie, A., Jumina, J., Mustofa, M., M, Hanafi, Kadidae, L. O., & I., Sahidin. (2017). Synthesis of 2-hydroxyxanthone from xanthone as a basic material for new antimalarial drugs. *Asian Journal Pharmaceutical and Clinical Research, 10*(12), 242-246. https://doi.org/10.22159/ajpcr.2017.v10i12.19858

Astalakshmi, N., Gokul, T., Gowri, S. K. B., Nandhini, M., Hari, H. S. M. R., Gowtham, S., Latha, S. T., and Kumar, S. (2022). Overview on molecular docking: A powerful approach for structure based drug discovery. *International Journal of Pharmaceutical Sciences Review and Research*, *77*(2), 180-198. https://dx.doi.org/10.47583/ijpsrr.2022.v77i02.029

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. CA: *A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Brikci-Nigassa, N. M., Nauton, L., Moreau, P., Mongin, O., Duval, R., Picot, L., Thiéry, V., Souab, M., Ruchaud, S., Bach, S., Guevel, R. L., Bentabed-Ababsa, G., Erb, W., Roisnel, T., Dorcet, V., & Mongin, F. (2020). Functionalization of 9-thioxanthone at the 1-position: From arylamino derivatives to [1]benzo(thio)pyrano[4, 3, 2- de]benzothieno [2,3-b]quinolines of biological interest. *Bioorganic Chemistry, 94*(1), 1-40. https://doi.org/10.1016/j.bioorg.2019.103347

Chemicalbook, 2016, *9-H-Thioxanthen-9-ol: Chemical Product Property*, https://www.chemicalbook.com/ChemicalProductProperty_EN_CB22151878.htm.

Chen, C., Chen, T., Lee, C., Shih, L., Lin, C., Hsieh, Y., Ali, A. A. A., & Huang, H. (2019). Synthesis and evaluation of the new 3-substituted-4-chloro-thioxanthone derivatives as potent anti-breast cancer agents. *Arabian Journal of Chemistry*, *12*(8), 3503-3516. https://doi.org/10.1016/j.arabjc.2015.10.010

Dawson, R. J. P., & Locher, K. P. (2006). Structure of a bacterial multidrug ABC transporter. *Nature*,

443(7108), 180–185. https://doi.org/10.1038/nature05155

Fatmasari, N., Kurniawan, Y. S. Jumina, J., Anwar, C., Priastomo, Y., Pranowo, H. D., Zulkarnain, A. K., & Sholikhah, E. N. (2022). Synthesis and in vitro assay of hydroxyxanthones as antioxidant and anticancer agents. *Scientific Reports, 12*(1), 1-8. https://doi.org/10.1038/s41598-022-05573-5

Ferreira, L.G., Dos Santos, R.N., Oliva, G., & Andricopulo, A.D. (2015). Molecular docking and structure-based drug design strategies. *Molecules, 20*(7), 13384-13421. https://doi.org/10.3390/molecules200713384

Fu, Y., Zhao, J., & Chen, Z. (2018). Insights into The molecular mechanisms of protein ligand interactions by molecular docking and molecular dynamics simulation: A case of oligopeptide binding protein. *Computational and Mathematical Methods in Medicine, 2018*(1), 1-12. https://doi.org/10.1155/2018/3502514

Hadanu, R. (2019). Modeling of quinoacridinium derivatives as antitumor agents using a QSAR analysis. *Indonesian Journal of Pharmacy, 30*(3), 167-179. https://doi.org/10.14499/indonesianjpharm30iss3pp167

Hermawan, F., Jumina, J., & Pranowo, H. D. (2020). Design thioxanthone derivatives as potential tyrosine kinase inhibitor: A molecule docking study. *Rasayan Journal of Chemistry*, *13*(4), 2626-2632. https://doi.org/10.31788/RJC.2020.1345699

Kumar, A., Roy, S., Tripathi, S., & Sharma, A. (2016). molecular docking based virtual screening of natural as potential BACE1 inhibitors: 3D QSAR pharmacore mapping and molecular dynamics analysis. *Journal of Biomolecular Structure & Dynamic*, *34*(2), 239-249. https://doi.org/10.1080/07391102.2015.1022603#abstract

Luanphaisarnnont, T. (2009). *Intermolecular Forces: van der Waals Interaction*, https://organicheskii.narod.ru/TOC/mezhmolekuljarnye_vzaimodejstvija.pdf

Male, Y., Sutapa, I. W., & Pusung, Y. A. D. (2018). Prediksi potensi antikanker senyawa turunan xanthon menggunakan hubungan kuantitatif struktur dam aktivitas (HKSA). *Chemistry Progress, 11*(1), 1-6. https://doi.org/10.35799/cp.11.1.2018.27907

Murthy, S.S. & Bala Narsaiah, T. (2019). Molecular docking studies of phytocompounds with transcriptional factors in hepatocellular carcinoma. *Rasayan Journal of Chemistry*, *12*(4), 2030–2038. https://doi.org/10.31788/RJC.2019.1245475

Narko, T., Benny, P., Riska, P., Dang, S., & Faridhatul, K. (2017). Molecular docking study of bulb of bawang dayak (*Eleutherine palmifolia* (L) Merr) compound as anti cervical cancer. *Jurnal Ilmiah Farmako Bahari*, *8*(2), 1-14. https://journal.uniga.ac.id/index.php/JFB

Palmeira, A., Vasconcelos, M. H., Paiva, A., Fernandes, M. X., Pinto, M., & Sousa, E. (2012). Dual inhibitors of p-glycoprotein and tumor cell growth: (re)discovering thioxanthones. *Biochemical Pharmacology*, *83*(1), 57-68. https://doi.org/10.1016/j.bcp.2011.10.004

Shi, Z., Chen, S., Xiao, Q. & Yin, D. (2021). Formation and disproportionation of xanthenols to xanthenes and xanthones and their use in synthesis. *The Journal of Organic Chemistry*, *86*(4), 3334-3343. https://doi.org/10.1021/acs.joc.0c02694

Sirait, P. S., Setyaningsih, I., & Tarman, K. (2019). Aktivitas antikanker ekstrak spirulina yang dikultur pada media walne dan media organik. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *22*(1), 50-59. https://doi.org/ 10.17844/jphpi.v22i1.25876

Ternay, A. L. & Chasar, D. W. (1967). The borohydride of thioxanthone sulfoxide. a base-induced dehydration of thioxanthenol sulfoxide. *The Journal of Organic Chemistry*, *32*(12), 3814-3817. https://doi.org/10.1021/jo01287a019

Wang, T., Wu, M. B., Chen, Z. J., Chen, H., Lin, J. P., & Yang, l. R. (2015). Fragment-based drug discovery and molecular docking in drug design. *Current Pharmaceutical Biotechnology*, *16*(1), 11-25. https://doi.org/10.2174/1389201015666141122204532

Zhao, X., H. Wanqui, Song, D., Lin, R., Huang, H., Huang, J., Wu, B., Huang, Y., & Ye, G. (2020). The hydrogen transfer reaction between the substance of triplet state thioxanthone and alkene with sp^3 hybridization hydrogen. *Journal of Molecular Modeling*, *26*(56), 1-8. https://doi.org/10.1007/s00894-020-4300-4