Effect of Hydroxyl Group on the Anticancer Activity of Xanthone Derivatives against Breast Cancer Cells

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Abstract*—Currently, breast cancer ranks among the deadliest diseases in both developed and developing nations. Consequently, it is urgent to discover active anticancer medication. Xanthone and its derivatives have been studied as anticancer agents because of their simple synthesis, ease of structural modification, and remarkable anticancer efficacy. This study assessed the effect of the number and position of the hydroxyl group on the xanthone structures on the anticancer activity against human breast cancer cells (T47D). Six hydroxyxanthones were synthesized through a one-pot reaction between benzoic acid and phenolic derivatives. Compared to xanthone, only 3-hydroxyxanthone, 1,3-dihydroxyxanthone, 3,6-dihydroxyxanthone, and 1,3,6-trihydroxyxanthone exhibit stronger anticancer activity. This result highlighted the importance of a hydroxyl group at the 3-position. The order of the anticancer activity of tetrahydroxyxanthone < dihydroxyxanthone < trihydroxyxanthone < monohydroxyxanthone except for 1-hydroxyxanthone. Of six hydroxyxanthones studied, the 3-hydroxyxanthone emerged as the most potent anticancer compound, exhibiting a half-maximal Inhibitory Concentration (IC_{50}) of 100.19 µM against the T47D cancer cell line. The hydroxyl group at the 3-position yielded stronger anticancer activity than the 1-position. On the other hand, monohydroxyxanthone had more potent anticancer activity than dihydroxyxanthone, trihydroxyxanthone, and tetrahydroxyxanthone. As the most potent anticancer agent, 3-hydroxyxanthone is non-toxic towards normal cell line (NIH3T3) with IC_{50} value and selectivity index of >1000 µg/mL and 51.27. These findings reveal an important knowledge on anticancer drug design and development based on xanthone derivatives.

***Keywords*—breast cancer, hydroxyl, synthesis, xanthone**

I. Introduction

As reported by the World Health Organization, cancer is recognized as the most lethal disease. It is estimated that one out of every six deaths is due to cancer. Approximately 13 million individuals were diagnosed with cancer in 2008. This number maintained an increase to 18 million in 2018 and is projected to hit 29 million by 2040 [1]. Nowadays, breast cancer is one of the top three causes of cancer-related deaths, attributed to its high mortality rate [2]. Lukasiewicz *et al.* [3] stated that in 2020, there were 2.3 million new cases of breast cancer diagnosed, and 666 thousand individuals passed away in the United States due to the disease that year. This report indicated that the breast cancer mortality rate had reached 29%, presenting a significant concern. As a result, breast cancer has been identified as a leading cause of mortality in certain developed and developing nations [4]. Furthermore, it is projected that total breast cancer mortality cases may rise to a higher number if the current death rate remains unchanged [5]. Consequently, there is no justification for not making a substantial effort to reduce the number of active breast cancer cases and its death rate in the future.

Several standard anticancer medications for the treatment and cure of breast cancer are now available, with doxorubicin as one of the most frequently utilized anticancer drugs [6]. Nevertheless, serious side effects of doxorubicin, such as hepatotoxicity and heart failure, have been documented recently and have proven its inefficacy for breast cancer treatment [7–9]. Moreover, some cancer cells have recently been resistant to doxorubicin treatment [10]. Researchers are giving their best effort into discovering new anticancer agents for breast cancer treatment. Thousands of anticancer compounds have been examined over the past several years. Among these anticancer compounds, xanthone and its derivatives demonstrate promising anticancer activities [11]. Due to its straightforward chemical structure, the xanthone derivative can interact with various key protein receptors, showing a broad range of anticancer spectrum influenced by the number, position, and type of functional groups attached [12].

Natural xanthones, i.e., mangostin, cudraxanthone, morusignin, and schomburgone are well-known anticancer compounds with low toxicity to normal cells [13, 14]. Their chemical structures contain at least one hydroxyl group. Mangostin has three hydroxyl groups at 1-, 3-, and 6-position. Cudraxanthone has two hydroxyl groups at 1- and 7-position. Morusignin has three hydroxyl groups at 1-, 4-, and 8-position. Besides, schomburgone has two hydroxyl groups at 1- and 6-position. Nonetheless, isolating these natural xanthones is a tedious process, as the yield often falls below 0.1%.

Hydroxyxanthone could be synthesized from an one-pot synthesis between benzoic acid and phenolic derivatives. This synthetic process offers a straightforward procedure and easy purification process with moderate to high synthetic yields [12]. However, there are few reports evaluating the number and position of the hydroxyl groups in xanthone derivatives concerning their anticancer activity against breast cancer line. Therefore, we evaluated the anticancer activities of the unmodified xanthone and six hydroxyxanthones to understand how the number and position of hydroxyl groups influence the anticancer efficacy against the breast cancer cell line. The hydroxyxanthones were prepared from the

benzoic acid and phenolic derivatives in an one-pot reaction. Afterward, their *in vitro* anticancer activity was performed against the breast cancer (T47D) cell line. Furthermore, an *in vitro* cytotoxicity assay towards the NIH3T3 normal cell line was conducted to calculate the selectivity index of the xanthone and hydroxyxanthones as the anticancer agent.

II. Materials and Methods

A. Materials

The salicylic acid ($C_7H_6O_3$), 2,4-dihydroxybenzoic acid ($C_7H_6O_4$), 2,3,4,5-tetrahydroxybenzoic acid ($C_7H_6O_6$), resorcinol ($C_6H_6O_2$), phloroglucinol ($C_6H_6O_3$), and Eaton's reagent (P_2O_5 in $MeSO_3H$) were provided by Merck in purity of higher than 95%. For the anticancer activity assay, dimethyl sulfoxide (DMSO, C_2H_6OS), phosphate buffer saline media, sodium dodecyl sulfate (SDS, $NaC_{12}H_{25}O_4S$), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, $C_{18}H_{16}BrN_5S$) were employed.

B. Instrumentations

The Fourier transform infrared (FTIR) spectrum of the synthesized products was recorded from a Shimadzu Prestige21 spectrophotometer. The Nuclear Magnetic Resonance (NMR) spectra of the synthesized compounds were measured from a JNM-ECZ500R/S1. The mass spectra of the title compounds were analyzed from a Shimadzu QP2010S instrument.

C. One-pot Synthesis of Hydroxyxanthones

Fig. 1 shows the synthesis scheme of hydroxyxanthones. Briefly, 0.015 mol of benzoic acid and 0.015 mol of phenolic derivatives were dissolved in 8 mL of Eaton's reagent. The mixture was heated at 353 K for 180 minutes. Then, the mixture was added to a crushed ice to isolate the crude product, and the crude product was purified using chromatography technique to yield the title compound.

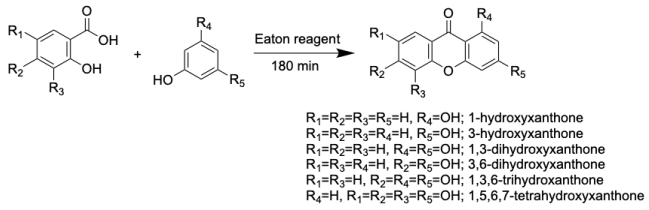

Fig. 1. The synthesis scheme of hydroxyxanthones.

D. Anticancer Activity and Cytotoxicity of Xanthone and Hydroxyxanthones

The anticancer activity of xanthone and six hydroxyxanthones was conducted against the T47D breast cancer cell line through an *in vitro* assay. At first, sterilization of all chemicals and equipment at 394 K was necessary. The T47D cancer cell line was provided by the Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. The cancer cells were cultured in phosphate buffer saline media at 310 K. The xanthone and hydroxyxanthones were dissolved at DMSO at 7.81–500 μM for each two-fold diluted concentration and placed in a 96-well microplate. The microplate was incubated at 310 K for 24 hours under a 5% carbon dioxide environment. Subsequently, 0.1 mL of MTT 5000 ppm was added and then the produced formazan was dissolved with the addition of 0.1 mL SDS 10% solution. The cell viability percentages were recorded by an ELISA reader at 595 nm. The half-maximal inhibitory concentration (IC_{50}) value was then obtained with the aid of probit analysis, and the selectivity index was calculated from the IC_{50} of cancer and normal cell lines. The data were subjected to statistical analysis to compare any significant difference using analysis of variance. The difference is considered significant at a p-value of less than 0.05.

III. Results and Discussion

A. One-pot Synthesis of Hydroxyxanthones

Six hydroxyxanthones, i.e, 1-hydroxyxanthone, 3-hydroxyxanthone, 1,3-dihydroxyxanthone, 3,6-dihydroxyxanthone, 1,3,6-trihydroxyxanthone, and 3,5,6,7-tetrahydroxyxanthone have been obtained as yellow solid in 6.6%, 30.2%, 46.5%, 21.9%, 15.4%, and 34.9%, respectively. Their FT-IR and NMR characterization data of the synthesized compounds are listed in Tables 1–2. The FT-IR characterization aims to elucidate the presence of the functional group in each hydroxyxanthone. The hydroxyl (O–H) group of hydroxyxanthone is observed as a broad signal at 3124–3503 cm^{-1}, whereas the carbonyl (C=O) group appears as a sharp signal at 1613–1621 cm^{-1}. On the other hand, the aromatic ring (C=C) of hydroxyxanthone is found at 1450–1489 cm^{-1}, whereas the phenoxy ether (C–O–C) group is detected at 1119–1180 cm^{-1}. It is found that the wavenumber for the C=O (1617±4 cm^{-1}), C=C (1469±20 cm^{-1}), and C–O–C (1150±30 cm^{-1}) signals are not significantly different because their chemical environment is similar. In contrast, the wavenumber for the O–H group varies from 3314±190 cm^{-1} depending on the presence of the hydrogen bond. No hydrogen bond is observed for 1-hydroxyxanthone and 3-hydroxyxanthone because they have only one O–H group; thus, the wavenumber for the O–H group is relatively low (3124–3325 cm^{-1}). One hydrogen bond is possible for 1,3-dihydroxyxanthone and 3,6-dihydroxyxanthone as they have two O–H groups; thus, the wavenumber for the O–H group is relatively higher (3325–3387 cm^{-1}). Meanwhile, 1,3,6-trihydroxyxanthone and 3,5,6,7-tetrahydroxyxanthone have two and three possible hydrogen bonds; thus, their wavenumber values are much higher (3503–3610 cm^{-1}).

Table 1. FT-IR characterization data of Hydroxyxanthones

Compound	Wavenumber (cm^{-1})			
	O–H	C=O	C=C	C–O
1-Hydroxyxanthone	3124	1613	1450	1119
3-Hydroxyxanthone	3325	1620	1489	1165
1,3-Dihydroxyxanthone	3387	1612	1458	1172
3,6-Dihydroxyxanthone	3325	1621	1458	1165
1,3,6-Trihydroxyxanthone	3503	1613	1458	1180
3,5,6,7-Tetrahydroxyxanthone	3610	1620	1450	1162

The ^{1}H-NMR and ^{13}C-NMR data, presented in Table 2, are utilized to characterize the proton and carbon of each hydroxyxanthone. Both 1-hydroxyxanthone and 3-hydroxyxanthone have seven aromatic protons (H_{Ar}) due to the presence of one hydroxyl group. Therefore, both of them

have seven H_{Ar} signals in the ^{1}H-NMR data. Specifically, 1-hydroxyxanthone shows four doublets (d) and three triplets (t) signals whereas 3-hydroxyxanthone shows four doublets (d), one doublet of doublet (dod) and two triplets (t) signals. On the other hand, 1,3-dihydroxyxanthone and 3,6-dihydroxyxanthone have six H_{Ar} due to the presence of two hydroxyl groups; thus, they show six H_{Ar} signals in the ^{1}H-NMR data. Specifically, 1,3-dihydroxyxanthone shows four doublets (d) and two triplets (t) signals, whereas 3,6-dihydroxyxanthone shows two doublets (d) and one doublet of doublet (dod) signals due to its symmetrical structure. The 1,3,6-trihydroxyxanthone has five H_{Ar} due to the presence of three hydroxyl groups; thus, it shows five H_{Ar} signals as four doublets (d) and one doublet of doublet (dod) in the ^{1}H-NMR data. The 3,5,6,7-trihydroxyxanthone has four H_{Ar} due to the presence of four hydroxyl groups; thus, it shows four H_{Ar} signals as two doublets (d) and two singlets (s) in the ^{1}H-NMR data.

Table 2. ^{1}H-NMR and ^{13}C-NMR characterization data of hydroxyxanthones

Xanthone	Chemical shift (ppm)		
	H_{Ar}	C=O	C_{Ar}
1-Hydroxy	6.8 (1H, d, 8 Hz), 7.0 (1H, d, 8 Hz), 7.4 (1H, t, 8 Hz), 7.6 (1H, d, 8 Hz), 7.7 (1H, t, 8 Hz), 7.8 (1H, t, 8 Hz), 8.3 (1H, d, 8 Hz)	183.8	108.3, 110.0, 111.4, 119.2, 121.8, 125.5, 126.9, 137.3, 138.4, 157.8, 157.9, 163.2
3-Hydroxy	6.9 (1H, d, 2 Hz), 7.0 (1H, dod, 2 Hz), 7.4 (1H, t, 2 Hz), 7.6 (1H, d, 2 Hz), 7.8 (1H, t, 8 Hz), 8.1 (1H, d, 8 Hz), 8.2 (1H, d, 8 Hz)	175.9	103.3, 114.7, 116.0, 118.7, 122.8, 124.9, 127.1, 129.2, 135.4, 157.1, 159.0, 164.6
1,3-Dihydroxy	6.2 (1H, d, 2 Hz), 6.4 (1H, d, 2 Hz), 7.4 (1H, t, 8 Hz), 7.5 (1H, d, 8 Hz), 7.8 (1H, t, 8 Hz), 8.2 (1H, d, 8 Hz)	181.8	95.4, 99.4, 103.9, 118.8, 121.8, 125.3, 126.6, 136.4, 157.5, 159.6, 164.9, 168.1
3,6-Dihydroxy	6.8 (2H, d, 2 Hz), 6.9 (2H, dod, 2 and 6 Hz), 8.1 (2H, d, 6 Hz)	177.7	103.3, 115.6, 115.9, 129.1, 159.9, 165.5
1,3,6-Trihydroxy	6.1 (1H, d, 2 Hz), 6.2 (1H, d, 2 Hz), 6.7 (1H, d, 2 Hz), 6.8 (1H, dod, 2 and 9 Hz), 8.0 (1H, d, 9 Hz)	179.9	93.7, 97.7, 101.8, 102.0, 112.9, 113.4, 126.7, 158.0, 158.1, 163.3, 164.4, 165.4
3,5,6,7-Tetrahydroxy	6.4 (1H, s), 6.5 (1H, d, 2 Hz), 6.8 (1H, s), 7.6 (1H, d, 2 Hz)	175.2	101.6, 102.2, 113.5, 114.1, 116.3, 128.5, 138.3, 138.7, 139.0, 139.1, 157.0, 165.9

The ^{13}C-NMR data of the hydroxyxanthones match well with the number of aromatic carbon (C_{AR}) in their chemical structure. As the presence of hydroxyl group does not change the number of C_{AR}, all hydroxyxanthones yielded twelve C_{AR} signals in their ^{13}C-NMR spectra. However, the hydroxyl group affects the chemical shift of the C_{AR} signals since the hydroxyl group causes a de-shielding effect that leads to a higher chemical shift. Specifically, 1-hydroxyxanthone and 3-hydroxyxanthone show C_{AR} signals in a range of 108.3–163.2 and 103.3–164.6 ppm, respectively. Both 1,3-dihydroxyxanthone and 3,6-dihydroxyxanthone give a higher chemical shift for C_{AR} signals, i.e., in a range of 95.4–168.1 and 103.3–165.5 ppm, respectively. The 1,3,6-trihydroxyxanthone gives a much higher chemical shift for C_{AR} signals, i.e., in a range of 93.7–165.4 ppm. The 3,5,6,7-tetrahydroxyxanthone shows the chemical shift of C_{AR} signals in a range of 101.6–165.9 ppm. The C=O signal for the hydroxyxanthones is observed at 175.2–183.8 ppm. Both FT-IR and NMR data of 1,3-dihydroxyxanthone, 3,6-dihydroxyxanthone, and 1,3,6-trihydroxyxanthone agree with the previous report by Bosson [15]. On the other hand, the mass spectra confirm the molecular ion (M^+) of 1-hydroxyxanthone and 3-hydroxyxanthone at *m/z* 212 for $C_{13}H_8O_3^+$. The mass spectra of 1,3-dihydroxyxanthone and 3,6-dihydroxyxanthone also match well with their molecular weight ($C_{13}H_8O_4$) by observing the M^+ signal at *m/z* 212. On the other hand, the M^+ signal of 1,3,6-trihydroxyxanthone ($C_{13}H_8O_5$) and 3,5,6,7-tetrahydroxyxanthone ($C_{13}H_8O_6$) are found at *m/z* 244 and 260, respectively. The chemical structure of the hydroxyxanthones is depicted in Fig. 2. These FT-IR, NMR, and mass spectra elucidation data support each other, concluding that all hydroxyxanthones have been successfully prepared.

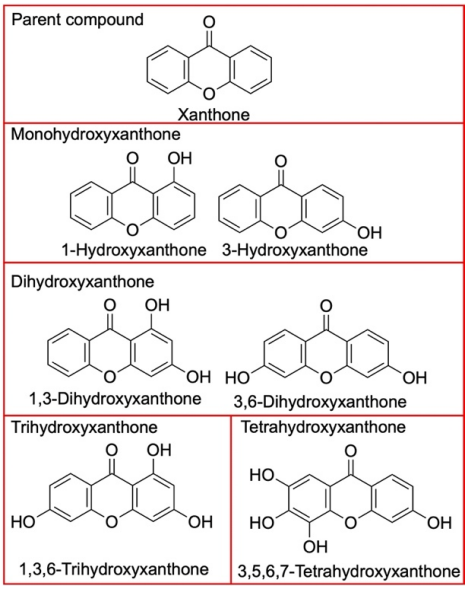

Fig. 2. The chemical structure of xanthone and hydroxyxanthones.

B. Anticancer Activity and Cytotoxicity of Xanthone and Hydroxyxanthones

The anticancer activity of hydroxyxanthones has been evaluated through an *in vitro* assay against T47D cancer cells. T47D cells are hormone-dependent epithelial breast cells [16]. These cancer cells are isolated from a pleural effusion of a 54-year-old cancer patient and they have been widely used in anticancer research [17]. The anticancer activity is expressed in the IC_{50} parameter. Lower IC_{50} represents a stronger anticancer activity as lower concentration is required to inhibit the growth of cancer cells [18]. The anticancer activity of xanthone and hydroxyxanthones is listed in Table 3. Xanthone gives an IC_{50} value of 194.34 μM. It is expected that the addition of hydroxyl group enhanced the anticancer activity of xanthone. However, the IC_{50} values of 1-hydroxyxanthone (IC_{50} = 248.82 μM) and 3,5,6,7-tetrahydroxyxanthone (IC_{50} > 1000

μM) are higher than xanthone (IC_{50} = 194.34 μM). On the other hand, 3-hydroxyxanthone (IC_{50} = 100.19 μM), 1,3-dihydroxyxanthone (IC_{50} = 137.24 μM), 3,6-dihydroxyxanthone (IC_{50} = 170.20 μM), and 1,3,6-trihydroxyxanthone (IC_{50} = 121.89 μM) exhibit lower IC_{50} values than xanthone (IC_{50} = 194.34 μM) as expected. All of these hydroxyxanthones have a hydroxyl group at 3-position, highlighting its importance for the anticancer activity against T47D cancer cells. However, the 3,5,6,7-tetrahydroyxanthone (IC_{50} > 1000 μM) generates a weaker anticancer activity than xanthone (IC_{50} = 194.34 μM) which could be caused by the effect of the hydroxyl group at 5-, 6-, and/or 7-position. A further study is required to clarify this finding.

The anticancer activity order of the hydroxyxanthones is as follows: 3,5,6,7-tetrahydroxyxanthone < 1-hydroxyxanthone < xanthone < 3,6-dihydroxyxanthone < 1,3-dihydroxyxanthone < 1,3,6-trihydroxyxanthone < 3-hydroxyxanthone. These results show that the anticancer activity of tetrahydroxyxanthone < dihydroxyxanthone < trihydroxyxanthone < monohydroxyxanthone in general, except for 1-hydroxyxanthone. Compared to doxorubicin as the positive control with an IC_{50} value of 60.35 μM against the T47D cell line [19], the anticancer activity of 3-hydroxyxanthone with an IC_{50} value of 100.19 μM is comparable.

Table 3. Anticancer activity of xanthone and hydroxyxanthones

Compound	IC_{50} (μM)*
Xanthone	194.34[a]
1-Hydroxyxanthone	248.82[b]
3-Hydroxyxanthone	100.19[c]
1,3-Dihydroxyxanthone	137.24[d]
3,6-Dihydroxyxanthone	170.20[e]
1,3,6-Trihydroxyxanthone	121.89[d]
3,5,6,7-Tetrahydroxyxanthone	> 1000[f]

*Different superscript letters show significant differences ($p < 0.05$) at the 5% significance test level.

The cytotoxicity of xanthone and hydroxyxanthones is shown in Table 4. Xanthone gave an IC_{50} value of 203.30 μM against the NIH3T3 normal cell line. Among six hydroxyxanthones, 1-hydroxyxanthone (IC_{50} = 22.42 μM) and 1,3-dihydroxyxanthone (IC_{50} = 66.58 μM) give lower IC_{50} values than xanthone (IC_{50} = 203.30 μM). It means both 1-hydroxyxanthone and 1,3-dihydroxyxanthone are more toxic than xanthone towards normal cell line. In contrast, 1,3,6-trihydroxyxanthone yields an IC_{50} value of 456.89 μM whereas 3-hydroxyxanthone, 3,6-dihydroxyxanthone, and 3,5,6,7-tetrahydroxyxanthone give high IC_{50} value (>1000 μM) demonstrating that these hydroxyxanthones are less toxic than xanthone. The cytotoxicity order of the hydroxyxanthones is as follows: 1-hydroxyxanthone < 1,3-dihydroxyxanthone < xanthone < 1,3,6-trihydroxyxanthone < 3-hydroxyxanthone = 3,6-dihydroxyxanthone = 3,5,6,7-tetrahydroxyxanthone. The data reveal that a hydroxyl group at the 3-position not only crucial for the anticancer activity enhancement but also important for the non-toxic profile of the xanthone derivatives. In contrast, a hydroxyl group at 1-position causes a toxic profile of hydroxyxanthone against the NIH3T3 cell line. This trend is supported from the IC_{50} values of 1-hydroxyxanthone, 1,3-dihydroxyxanthone, and 1,3,6-trihydroxyxanthone (IC_{50} = 22.42–456.89 μM) that are lower than 3-hydroxyxanthone, 3,6-dihydroxyxanthone, and 3,5,6,7-tetrahydroxyxanthone (IC_{50} > 1000 μM). Compared to doxorubicin as the positive control with an IC_{50} value of 11.44 μM against the NIH3T3 cell line, the 3-hydroxyxanthone with an IC_{50} value of higher than 1000 μM is less toxic to normal cells.

Table 4. Cytotoxicity of xanthone and hydroxyxanthones

Compound	IC_{50} (μM)*	Selectivity index*
Xanthone	203.30[a]	1.05[a]
1-Hydroxyxanthone	22.42[b]	0.09[b]
3-Hydroxyxanthone	> 1000[c]	51.27[c]
1,3-Dihydroxyxanthone	66.58[b]	0.49[b]
3,6-Dihydroxyxanthone	> 1000[c]	17.03[d]
1,3,6-Trihydroxyxanthone	456.89[d]	3.75[e]
3,5,6,7-Tetrahydroxyxanthone	> 1000[c]	0.97[a]

*Different superscript letters show significant differences ($p < 0.05$) at the 5% significance test level.

The toxicity of anticancer agents is also reflected by the selectivity index parameter. A higher selectivity index shows a lower toxicity profile. The selectivity index of xanthone is found to be 1.05, while 1-hydroxyxanthone, 1,3-dihydroxyxanthone, and 3,5,6,7-tetrahydroxyxanthone gave a selectivity index of 0.09, 0.49 and 0.97, respectively. This result shows that either 1-hydroxyxanthone or 1,3-dihydroxyxanthone or 3,5,6,7-tetrahydroxyxanthone is more toxic than xanthone; thus, they are not recommended as the anticancer agent. Nevertheless, 1,3,6-trihydroxyxanthone, 3,6-dihydroxyxanthone, and 3-hydroxyxanthone yield a selectivity index of 3.75, 17.03, and 51.27, respectively. The toxicity order of the hydroxyxanthones is as follows: 1-hydroxyxanthone < 1,3-dihydroxyxanthone < 3,5,6,7-tetrahydroxyxanthone < xanthone < 1,3,6-trihydroxyxanthone < 3,6-dihydroxyxanthone < 3-hydroxyxanthone. Compared to doxorubicin as the positive control with a selectivity index of 0.19, the 3-hydroxyxanthone with a selectivity index of 51.27 has a lower toxicity profile, which is remarkable.

IV. Conclusion

Six hydroxyxanthones, i.e., 1-hydroxyxanthone, 3-hydroxyxanthone, 1,3-dihydroxyxanthone, 3,6-dihydroxyxanthone, 1,3,6-trihydroxyxanthone, and 3,5,6,7-tetrahydroxyxanthone have been successfully synthesized in this work in 6.6%–46.5%. Their chemical structures have been confirmed through spectroscopic characterization data. The *in vitro* anticancer and cytotoxicity assays reveal that a hydroxyl group at the 3-position not only crucial for the anticancer activity enhancement against T47D cells but also important for the non-toxic profile of xanthone towards NIH3T3 cells. Among the evaluated hydroxyxanthones, 3-hydroxyxanthone yields the lowest IC_{50} value against T47D cancer cells and the highest IC_{50} value towards NIH3T3 normal cell lines. This result shows that 3-hydroxyxanthone is the most potent anticancer agent for breast cancer cells, with a non-toxic profile for normal cells.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Y. S. K conducted the research, analyzed the data, and wrote the paper; E. N. S. gave supervision on the anticancer assay and supported the data analysis; J. and H. D. P. gave supervision on the organic synthesis, provided the chemicals, and supported the data analysis; all authors had approved the final version.

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