Identification of Novel Sesamol Dimers with Unusual Methylenedioxy Ring-Opening Skeleton and Evaluation of Their Antioxidant and Cytotoxic Activities

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Abstract: Background: Sesamol is a widely used antioxidant for the food and pharmaceutical industries. The oxidation products of this compound may be accumulated in foods or ingested. Little is known about its effect on human health.

Objective: It is of great interest to identify the oxidation products of sesamol that may be beneficial to humans. This study was undertaken to identify the oxidation products of sesamol and investigate their antioxidant and cytotoxic activities.

Materials and Methods: Using the ferricyanide oxidation approach, four oxidation products of sesamol (2, 3, 20 & 21) have been identified. Structural elucidation of these compounds was established on the basis of their detailed NMR spectroscopic analysis, mass spectrometry and x-ray crystallography. Additionally, a formation mechanism of compound 20 was proposed based on high-resolution mass spectrometry-fragmentation method. The antioxidant activities of these compounds were determined by the DPPH, FRAP, and ABTS assays. The in vitro antiproliferative activity of these compounds was evaluated against a panel of human cancer cell lines as well as non-cancerous cells.

Results: Two oxidation products of sesamol were found to contain an unusual methylenedioxy ring-opening skeleton, as evidenced by spectroscopic and x-ray crystallographic data. Among all compounds, 20 displayed impressive antiproliferative activities against a panel of human cancer cell lines yet remained non-toxic to non-cancerous cells. The antioxidant activities of compound 20 are significantly weaker than sesamol as determined by the DPPH, FRAP, and ABTS assays.

Conclusion: The oxidation products of sesamol could be a valuable source of bioactive molecules. Compound 20 may be used as a potential lead molecule for cancer studies.

Keywords: Bioactive compounds, structure elucidation, sesamol, oxidation, antioxidant, cytotoxic activity.

1. INTRODUCTION

Sesamol has recently attracted much attention as a potent antioxidant for food and pharmaceutical industries [1]. Its antioxidant action is to donate hydrogen to reactive radical species [2], and the resulting sesamolyl radical may dimerize or form a diverse range of oxidation products including quinones (Fig. 1) [1-6]. Interestingly, a number of these oxidation products have been found to exhibit anticancer activity. Notably, compound 5 was cytotoxic against human leukemia K562 cells and non-proliferative rat thymocytes [7]. Compound 7 showed both proapoptotic and inhibitory action on late stage of apoptosis in rat thymocytes [8]. The quinone-type of oxidation products are also potentially valuable anticancer agents [9, 10].

However, the identification of these oxidation products of sesamol in food is difficult because they are often present in minute quantities [11]. Hence, the oxidation of sesamol has been carried out in the presence of an oxidant or oxidizing agent. A number of oxidants have been used for this purpose, including ferricyanide [2], ferric chloride [3, 4], laccase [1] or peroxydisulfate enzyme [12], persulfate reagent [5], hypervalent iodine reagent [13], and metal phenolato [14]. The oxidation of sesamol can also be carried out with electrochemical oxidation method [6, 15-17]. In most cases, dimer 2 is formed predominantly from the reaction. In this study, we reported the synthesis and structural characterization of new
dimeric products from ferricyanide oxidation of sesamol and the antioxidant activity, as well as the cytotoxicity of these compounds.

2. MATERIALS AND METHOD

2.1. General

The chemicals sesamol, iron(III) chloride hexahydrate, and potassium ferricyanide(III) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane, deuterated chloroform (CDCl₃-d₆), deuterated dimethyl sulfoxide (DMSO-d₆), ethyl acetate, n-hexane, tetrahydrofuran and toluene were purchased from Merck (Darmstadt, Germany) and were of analytical grade. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminium silica gel sheets (Kieselgel 60 F254). Column chromatography was performed with silica gel 60 (230-400 mesh) from Merck. All melting points were taken on a Stuart melting point apparatus SMP30 (Staffordshire, UK). NMR spectra were acquired at 20°C using Jeol ECA 400 (400 MHz) (Akishima, Tokyo, Japan) and Bruker Avance III HD (600 MHz) (Billerica, USA). NMR spectrometers with tetramethylsilane as the internal standard. All chemical shifts are reported in ppm, and J-couplings are reported in Hz. MS analysis was performed on an Agilent 6500 series-accurate mass Q-TOF (Santa Clara, CA, USA).

2.2. Procedure for the Oxidation of Sesamol

Sesamol (1) (0.5 mmol) was dissolved in methanol (2.5 mL). Potassium ferricyanide (K₃Fe(CN)₆, 0.5 mmol) in 2.5 mL of water was added to this solution and the reaction mixture was rubber-stoppered and stirred for 2-24 h (Table 1 and supplementary information). The suspension was extracted with EtOAc (3 × 10 mL). The combined organic layers were evaporated under reduced pressure to give a brown solid as the crude product. The crude product was purified by column chromatography over silica gel using hexane and EtOAc (7:3-1:1 v/v) as the mobile phase to afford the corresponding products.

2.2.1. 9a-hydroxy-5a,9-dimethoxy-5a,6,9,9a-tetrahydrobenzof[b][1,3]dioxolo[4,5-f]benzofuran-7(8H)-one (21)

Colorless solid. M.p = 131–133 °C. Optical activity = 0 °C (ATR, v cm⁻¹): 1713 (C=O), 1462 (COCH₂-), 1293 (C-O). ¹H-NMR (400 MHz, CDCl₃): δ 6.83 (s, 1H), 6.33 (s, 1H), 5.91 (d, J = 1.2 Hz, 1H), 5.90 (d, J = 1.2 Hz, 1H), 3.88 (dd, J = 10.2, 3.6 Hz, 1H), 3.60 (s, 1H), 3.43 (s, 6H), 3.12 (d, J = 16.2 Hz, 1H), 2.76 (d, J = 16.2 Hz, 1H), 2.62 (dd, J = 18.0, 3.6 Hz, 1H), 2.19 (dd, J = 18.0, 10.2 Hz, 1H). ¹³C-NMR (100 MHz, CDCl₃): δ 204.07, 152.15, 149.68, 143.21, 119.10, 108.20, 106.43, 101.85, 93.72, 83.47, 80.56, 59.08, 51.00, 43.19, 40.56. HRMS (ESI): m/z [M + H⁺] calculated for C₂₁H₁₉O₇: 309.0974; found: 309.0968.

2.2.2. 9-methoxybenzof[b][1,3]dioxolo[4,5-f]benzofuran-7(8H)-one (21)

Brown amorphous. M.p = 180-182°C. IR (ATR, v cm⁻¹): 1276 (C-O). ¹H-NMR (400 MHz, DMSO-d₆): δ 9.83 (s, 1H), 7.27 (d, J = 6.6 Hz, 2H), 6.58 (d, J = 1.7 Hz, 1H), 6.38 (d, J = 1.5 Hz, 1H), 6.07 (2H), 3.93 (s, 3H). ¹³C-NMR (100 MHz, DMSO-d₆): δ 158.35, 158.26, 155.20, 149.94, 145.82, 144.19, 116.64, 105.76, 101.84, 100.39, 94.76, 94.37, 91.27, 56.05. HRMS (ESI): m/z [M - H⁻] calculated for C₁₄H₁₀O₅: 257.0455; found: 257.0401.

2.3. Cell Lines and Cell Culture

The human breast cancer cells (MDA-MB-231, MDA-MB-468, HCC38 and MCF7), nasopharyngeal cancer cells (HK1, CNE1, SUNE1 and TW01), lung cancer cells (H1299, H23, Calu-1 and A549), colorectal cancer cells (HCC2998, SW48, HT29 and HCT116) and endometrial cancer cells (RL95, AN3CA, HEC-1A and HEC-1B) were maintained in RPMI 1640 medium supplemented with 10% FBS (Bovine Serum Albumin) and 100 µg/mL of streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The normal human endometrial cells (T-HEC) and lung cells (MRC5) were maintained in Keratinocyte-SFM containing Epidermal Growth Factor (EGF 1-53) and Bovine Pituitary Extract (BPE) (Invitrogen, USA). All cells were maintained at 37°C under 5% CO₂ in a humidified incubator.

Fig. (1). Oxidation products of sesamol.
2.4. Cell Proliferation Assay

The *in vitro* cell line primary screen was conducted using Cell Titer-Glo® luminescent assay. Briefly, a total of 1500 cells/well were seeded overnight in a 384-well plate and treated with the designated compound at 100 μM for 72 hours. Cell Titer-Glo® Reagent was added in each well and further incubated for 10 minutes. Finally, the luminescent signal was recorded by a CCD camera. The results were compiled as the percentage of the inhibition of cell proliferation. The primary screen utilized 20 different human cancer cell lines. In order to further assess the selectivity of the isolated compounds towards cancerous and non-cancerous cells, the above process was repeated on the non-cancerous cells (T-HESC and MRC5).

2.5. 1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging Activity (DPPH) Assay

DPPH free radical scavenging activity was measured according to the method of Brand-Williams [18] with some modifications. Firstly, a DPPH solution (100 μM) was freshly prepared using methanol. Then, an aliquot of DPPH solution (195 μl) was mixed with 50 μl of the sample compounds (20 and sesamol). Antioxidants including gallic acid, ascorbic acid and 2,6-di-tert-butyl-4-methylphenol (BHT) were used as positive controls. The mixture was incubated for 30 minutes before the absorbance was read at 515 nm. All compounds were tested at five different concentrations (0–100 μM). Data were calculated and expressed in percentage (%) using the following equation:

\[
\text{DPPH radical scavenging activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample or standard}})}{A_{\text{control}}} \times 100
\]

where; \( A_{\text{control}} \) = absorbance of DPPH radicals without sample or standard; \( A_{\text{sample or standard}} \) = absorbance of DPPH radicals with sample or standard. The concentration of samples and standards that inhibits 50% of the DPPH radicals (IC50) was calculated and expressed as μM.

2.6. ABTS Radical Cation Decolorization Assay

ABTS radical cation decolorization assay was determined using 2,2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) [19]. Initially, a stock solution contained 7 mM ABTS® and 2.45 mM potassium peroxodisulfate was prepared in 10 ml of distilled water. The solution was then incubated for 12-16 hours in the dark at room temperature (25°C). Then, the stock solution was further diluted to obtain an absorbance of 0.70 ± 0.05 at 734 nm. An aliquot of ABTS solution (300 μl) was mixed with 3 μl of the sample compounds (20 and sesamol) or positive controls (100 μM). The mixture was incubated for 6 minutes before the absorbance was taken at 734 nm. Gallic acid, ascorbic acid and BHT were used as positive controls. Antioxidant capacity was calculated in percentage as the following equation:

\[
\text{Antioxidant capacity (\%)} = \frac{(A_{\text{ABTS}} - A_{\text{sample or standard}})}{A_{\text{ABTS}}} \times 100
\]

where; \( A_{\text{ABTS}} \) = absorbance of ABTS radicals without sample or standard; and \( A_{\text{sample or standard}} \) = absorbance of ABTS radicals with sample or standard. The values of Trolox equivalent antioxidant capacity (TEAC) were calculated based on the calibration curve plotted using Trolox (0–1.6 mM). Data were reported as mmol Trolox equivalents (TE)/g compound.

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was conducted using the method of Benzie and Strain [20] with slight modifications. FRAP reagent was first prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripryridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl) and 20 mM iron chloride (FeCl3) at a ratio of 10:1:1 (v:v:v), respectively. Then, an aliquot of FRAP reagent (300 μl) was added with 5 μl of 100 μM sample compounds (20 and sesamol) or positive controls (gallic acid, ascorbic acid and BHT). The mixture was incubated for 30 min at 37°C before the absorbance was taken at 595 nm. Iron sulfate (FeSO4) (0.1 mM) was used to plot the standard calibration curve. Results were calculated using the calibration curve and expressed as mmol Fe2+/g compound.

2.8. Statistical Analysis

All data were reported as mean ± standard deviation from minimum of three independent experiments. Statistical significant differences were analyzed using one-way analysis of variance or independent sample t test through SPSS (version 18.0) for Windows. A \( p \) value less than 0.05 (\( p < .05 \)) was considered a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. Synthesis

Initially, the oxidation of sesamol was carried out with FeCl3 in methanol at 40°C for 24 hours and the reaction was continually monitored on a daily basis for up to 8 days according to the protocol of Masuda (entry 1, Table 1) [3]. However, in this reaction, only a mixture of unidentified products was observed. Apart from FeCl3, the reaction was also examined with ferricyanide(K3Fe(CN)6), TEMPO, and persulfate (K2S2O8) (entries 2-9, Table 1). Among them, ferricyanide was the most promising oxidant, affording products 2, 3, 20 and 21 in 5-30% yield (entry 4, Table 1) and 90% conversion. Interestingly, when the ferricyanide oxidation of sesamol was carried out in the water, only dimer 2 and trimer 3 were observed (entry 3, Table 1). No product was observed for the ferricyanide oxidation of sesamol in methanol and starting material was recovered (entry 2, Table 1). TEMPO was less effective, affording only dimer 2 in 20% yield (entry 6, Table 1). While the coupling reaction with two equivalent of K2S2O8 in CCl4-COOH was reported to afford dimer 2 in 70% yield [5], the reaction did not proceed in methanol, water, or mixture of acetonic and water (1:1 v/v) (entries 7-9, Table 1 and supplementary information). The oxidation reaction using base (i.e. K2CO3, NaOH) has resulted in sample decomposition (entries 5 and 9, Table 1).

3.2. Structural Elucidation

The structures of the known compounds 2 and 3 were determined spectroscopically and matched with literature data [1]. Compound 20 was isolated as colorless cubic crystals, which turned black and decomposed after having been left at room temperature for two weeks. The HR-ESI-MS of 20 (m/z 309.0968 [M+H]+) suggested \( C_{11}H_{16}O_2 \) as the molecular formula of 20. The 1H NMR of 20 showed two singlets corresponding to two aromatic protons (δ 6.83 and 6.33), two doublets corresponding to a methylenedioxy group (δ 5.90 and 5.91, J = 1.2 Hz) and a singlet representing two oxygenated protons (δ 7.34). There were also chemical shifts indicating the presence of a methine group (δ 3.88, dd, J = 10.2, 3.6 Hz, H5′) and a methylene group (δ 3.12, d, J = 16.2 Hz, H2′,8′, and δ 2.76, d, J = 16.2 Hz, H2′,8′). The doublet of doublets at δ 2.62 (J = 18.0, 3.6 Hz, H6′), and δ 2.19 (J = 18.0, 10.2 Hz, H6′) were assigned to another methylene group, while the singlet at δ 3.60 was assigned to a hydroxy group (Fig. 2a). The IR and 13C NMR spectra indicated the presence of a carbonyl group in 20.

The methoxy groups were attached at the 3′ and 5′-positions of 20, as determined by the HMBC data (Fig. 2b). The HMBC data also indicated that the 3′-position should be an acetal carbon by considering its downfield carbon chemical shift (δ 108.20). The 4′-position was substituted by an OH group, which was deduced from the chemical shift value of C4′ (δ 83.47). The HMBC correlations between this OH (δ 3.60) and C5′, as well as between H5 (δ 6.83) and C6′, suggested C5′H16O2 as the molecular formula of 20. The 1H NMR of 20 showed two singlets corresponding to two aromatic protons (δ 6.83 and 6.33), two doublets corresponding to a methylenedioxy group (δ 5.90 and 5.91, J = 1.2 Hz) and a singlet representing two oxygenated protons (δ 7.34). There were also chemical shifts indicating the presence of a methine group (δ 3.88, dd, J = 10.2, 3.6 Hz, H5′) and a methylene group (δ 3.12, d, J = 16.2 Hz, H2′,8′, and δ 2.76, d, J = 16.2 Hz, H2′,8′). The doublet of doublets at δ 2.62 (J = 18.0, 3.6 Hz, H6′), and δ 2.19 (J = 18.0, 10.2 Hz, H6′) were assigned to another methylene group, while the singlet at δ 3.60 was assigned to a hydroxy group (Fig. 2a). The IR and 13C NMR spectra indicated the presence of a carbonyl group in 20. The methoxy groups were attached at the 3′ and 5′-positions of 20, as determined by the HMBC data (Fig. 2b). The HMBC data also indicated that the 3′-position should be an acetal carbon by considering its downfield carbon chemical shift (δ 108.20). The 4′-position was substituted by an OH group, which was deduced from the chemical shift value of C4′ (δ 83.47). The HMBC correlations between this OH (δ 3.60) and C5′, as well as between H5 (δ 6.83) and C6′.
Table 1. Oxidation of sesamol under different reaction conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidant (equiv)</th>
<th>Solvent</th>
<th>Base (equiv)</th>
<th>(T (\degree C))</th>
<th>Time (h)</th>
<th>Conv (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{c,d})</td>
<td>(\text{FeCl}_3 (0.1))</td>
<td>MeOH</td>
<td>–</td>
<td>40</td>
<td>8 days</td>
<td>95</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>(\text{K}_3\text{Fe(CN)}_6 (1))</td>
<td>MeOH</td>
<td>–</td>
<td>28</td>
<td>24</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>(\text{K}_3\text{Fe(CN)}_6 (1))</td>
<td>(\text{H}_2\text{O})</td>
<td>–</td>
<td>28</td>
<td>24</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>(\text{K}_3\text{Fe(CN)}_6 (1))</td>
<td>MeOH: (\text{H}_2\text{O} (1:1 \text{ v/v}))</td>
<td>–</td>
<td>28</td>
<td>24</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>(\text{K}_3\text{Fe(CN)}_6 (1))</td>
<td>MeOH: (\text{H}_2\text{O} (1:1 \text{ v/v}))</td>
<td>NaOH (1)</td>
<td>28</td>
<td>2</td>
<td>/</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>TEMPO (1)</td>
<td>MeOH: (\text{H}_2\text{O} (1:1 \text{ v/v}))</td>
<td>–</td>
<td>60</td>
<td>24</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>(\text{K}_3\text{S}_2\text{O}_8 (2))</td>
<td>MeOH</td>
<td>–</td>
<td>28</td>
<td>24</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>(\text{K}_3\text{S}_2\text{O}_8 (2))</td>
<td>(\text{H}_2\text{O})</td>
<td>–</td>
<td>28</td>
<td>24</td>
<td>/</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>(\text{K}_3\text{S}_2\text{O}_8 (2))</td>
<td>MeOH</td>
<td>K$_3$CO$_2$ (1)</td>
<td>28</td>
<td>2</td>
<td>/</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^{c}\)Reaction conditions: 1 (0.5 mmol), solvent (5.0 mL), r.t. (28 \(\degree C\)).

\(^{d}\)Conversion was based on \(^1\)H NMR analyses.

\(^{e}\)Isolated yield.

\(^{f}\)Under oxygen atmosphere.

\(^{g}\)Mixture of unidentified compounds.

\(^{h}\)Decomposition.

Fig. (2). (a) Key C-H long-range correlations (arrows) observed in HMBC of compound 20. (b) Key NOE correlations observed in NOESY (dashed line) and NOE differential spectra (arrows) of 20. The stereo structure of 20 was optimized by Frog2 program.

and C4' prove the position of 4'-OH group. A typical vicinal coupling constant \(^3J_{HH}\) of H5' (3.6, 10.2 Hz) along with the HMBC correlations between H5' and C6, C1', C3', and 5'-methoxy group arrange H5' and H6' on the cyclohexanone ring. The HMBC correlations between H6' and C2' and between H2' and C6' establish the 2',6'-position of H2' and H6' on the cyclohexanone ring.

The stereochemistry of 20 was elucidated by analysis of the NOE correlations, as shown in Fig. (2c). The NOE differential spectra showed a significant enhancement of H2'\(^{\beta}\) (4%) and H6'\(^{\beta}\) (5%) signals when H5' was irradiated. The NOESY showed a correlation between H2'\(^{\beta}\) and H2'\(^{\alpha}\). The structure of 20 was confirmed unambiguously by single-crystal X-ray crystallography (Fig. 2d and supplementary information).

The ESI-MS spectra of 20 showed the [M+H]\(^{+}\) ion and a minor [M+Na]\(^{+}\) ion. The fragmentations of the [M+H]\(^{+}\) ion were induced by collision with nitrogen gas under tandem mass spectrometry conditions (ESI-MS/MS) and the main product ions observed are presented in Scheme 1. A loss of 32 Da (CH$_3$OH) was observed in 20 to give an intense product ion at m/z 277 [M+H− CH$_3$OH]$^+$. Subsequent removal of CH$_3$OH could account for the product ion with m/z 245, corresponding to the ion [M+H−2CH$_3$OH]$^+$. Finally a
Compound dehydration and conjugate addition of methylenedioxy group of tetraacetate methylenedioxy ring cleavage has been reported by using lead methylenedioxy ring cleavage to afford diol. Subsequently, the intermediate furnishes in species ferricyanide oxidation to the resonance stabilized sesamolyl radical.

3.3. Formation Mechanism of \( 20 \)

Therefore, structure \( 21 \) was determined by the HMBC data to be at the 5′-position. The IR and \(^{13}\)C NMR spectra of \( 21 \) indicated the absence of a carbonyl group. Therefore, structure \( 21 \) for the benzofuran was apparent.

3.3. Formation Mechanism of \( 20 \)

A possible mechanism for the formation of \( 20 \) from sesamol \( 1 \) is depicted in Scheme 2. We propose that sesamol undergoes ferricyanide oxidation to the resonance stabilized sesamolyl radical species \( 1a \) and \( 1b \), which dimerize and protonate to give intermediate \( 6 \). Conjugate addition of methanol to compound \( 6 \) furnishes intermediate \( 6a \), which mimics analogues \( 7 \) and \( 8 \) [4]. Subsequently, the intermediate \( 6a \) undergoes ferricyanide-mediated methylenedioxy ring cleavage to afford diol \( 6b \). A similar methylenedioxy ring cleavage has been reported by using lead tetaacetate [21]. We hypothesize that the C3′- and C4′-connected methylenedioxy group of \( 6a \) would be prone to cleavage. Finally, dehydration and conjugate addition of \( 6b \) with methanol produce compound \( 20 \).

3.4. Cytotoxic and Antioxidant Activities

The in vitro antiproliferative activity of all isolated compounds (2, 3, 20 and 21) and positive control paclitaxel was evaluated against a panel of human breast (MDA-MB-231, MDA-MB-468, MCF-7, HCC38), endometrial (RL95, AN3CA, HEC-1B, HEC-1A), colorectal (HT29, SW48, HCT116, HCC2998), nasopharyngeal (TW01, HK1, SUNE1, CNE1), lung (H1299, H23, A549, Calu-1) cancer cell lines and human non-cancerous endometrial cells (T-HESC) as well as non-cancerous lung cells (MRC5). All cells were cultured based on our previous studies [22, 23]. Cells were treated with 100 μM of the tested compounds for 72 hours. The cell viability was quantified using previously established CellTiter-Glo® Luminescent Cytotoxicity Assay [24, 25] (Fig. 3). Among all tested compounds, \( 20 \) displayed impressive antiproliferative activities against all tested cancer cell lines as indicated in Fig. (3). Compounds 2, 3, and 21 were less potent compared to compound \( 20 \). It is noteworthy that the cell viability of compound \( 20 \) in cancer cells was much lower than that of non-cancerous cells (MRC5 and T-HESC), indicating that \( 20 \) was selective towards cancer cells (Fig. 4). Although compounds \( 20 \) and 21 were structurally quite similar, their antiproliferative activities were significantly different. However, \( 20 \) was unstable in solution and decomposed within hours. Further investigation on deducing the inhibition concentration (IC\(_{50}\)) was halted.

To investigate whether the cytotoxicity of \( 20 \) correlates with its antioxidant activity, we examined the antioxidant activity of \( 20 \) by using DPPH, FRAP, and ABTS assays. Ascorbic acid, gallic acid, and 2,6-di-tert-butyl-4-methylphenol (BHT) were used as positive controls and the results are presented in Fig. (5) and Table 2.
Fig. (3). Cell viability of compounds 2, 3, 20 and 21 against a panel of human cancer cells and non-cancer cells at a concentration of 100 µM at 72 hours. Cell TiterGlo® luminescent assay was used to quantify cell viability. Results were the mean ± S.D. from at least three independent experiments.

Fig. (4). Cell viability of compound 20 against human cancer cell lines and non-cancer cells at a concentration of 100 µM at 72 hours. Results were the mean ± S.D. from at least three independent experiments.

The DPPH assay was used to evaluate the scavenging ability of 20 on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and was compared with sesamol and the positive controls. The DPPH radical scavenging efficiency was in the order: gallic acid > sesamol > ascorbic acid > BHT > 20 with lower IC_{50} indicating stronger antioxidant efficiency. As illustrated in Fig. (5), the DPPH radical scavenging activity of 20 was lower compared to gallic acid, sesamol and ascorbic acid, but it was slightly higher than BHT at doses between 6.25–50 µM. At the low concentration range (6.25-12.5 µM), gallic acid showed significant changes in the antioxidant activities. However, the changes in the antioxidant activities of 20 were not significantly observed at these concentrations. Hence, the curve was not dose-dependent at 6.25-12.5 µM in Fig. (5). This phenomenon can be explained because gallic acid is a stronger antioxidant in DPPH radical scavenging assay. The antioxidant activity of 20 was 5-folds weaker than gallic acid (17.5% compared...

To 83.9% of gallic acid). No IC₅₀ value was reported for 20 and BHT as both compounds were not active up to 100 µM.

The FRAP assay was used to measure the ability of 20 to reduce a ferric tripyridyltriazine (Fe³⁺–TPTZ) complex to ferrous tripyridyltriazine (Fe²⁺–TPTZ). In this assay, 20 showed a lower FRAP value by 6-folds compared to sesamol (Table 2). The ability of 20 to reduce a ferric tripyridyltriazine (Fe³⁺–TPTZ) complex was the lowest amongst all tested samples.

The ABTS assay was used to determine the Trolox equivalent antioxidant capacity (TEAC) of 20. In this assay, 20 inhibited 3.6% of the ABTS⁺ radicals at a concentration of 100 µM. The value for the inhibitory activity is lower when compared with the sesamol (12.8%). The antioxidant capacity, as measured by the ABTS assay, followed the order: gallic acid > sesamol > ascorbic acid > BHT > 20, with antioxidant capacity of 354.8, 295.1, 115.4, 83.8 and 25.2 mmol Trolox equivalents/g compound, respectively (Table 2).

Overall, the results indicated that 20 is a weak antioxidant. The present data showed that the antioxidant activity of 20 is not corroborated with its molecular structure. Although this compound has a hydroxy group and a benzodioxole group similar to that of sesamol, its antioxidant activity is significantly weaker than sesamol. We reasoned that the poor stability of 20 in solution may account for the weak antioxidant activity.

CONCLUSION

In conclusion, sesamol is easily oxidized to produce a variety of oxidation products. In this study, ferricyanide oxidation was found to be an effective method to produce oxidation products of sesamol. The structural characterization of four dimeric products was achieved by NMR spectroscopic methods and mass spectrometry. A formation mechanism of compound 20 was proposed involving selective methylenedioxy ring cleavage. Although compound 20 displayed promising cytotoxic activities against human cancer cells, it decomposed easily in solution. The antioxidant activities of 20 were weaker than sesamol as determined by the DPPH, FRAP and ABTS assays. Studies are presently underway to evaluate the toxicity of these oxidation products.

Table 2. Antioxidant activities of 20 as determined by the DPPH, FRAP, and ABTS methods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH</th>
<th>FRAP</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antioxidant Activity(%)</td>
<td>IC₅₀ (µM)</td>
<td>µM Fe²⁺ at 100 µM</td>
</tr>
<tr>
<td>20</td>
<td>17.46 ± 0.22</td>
<td>&gt; 100</td>
<td>44.83 ± 12.83</td>
</tr>
<tr>
<td>Sesamol</td>
<td>57.63 ± 3.44</td>
<td>86.31 ± 6.40</td>
<td>292.39 ± 18.65</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>53.41 ± 0.35</td>
<td>94.17 ± 0.77</td>
<td>247.86 ± 11.08</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>83.89 ± 0.89</td>
<td>19.44 ± 0.40</td>
<td>796.17 ± 14.13</td>
</tr>
<tr>
<td>BHT</td>
<td>36.71 ± 1.49</td>
<td>&gt; 100</td>
<td>158.30 ± 12.71</td>
</tr>
</tbody>
</table>

*Antioxidant activity (%) at 100 µM. Values are given as mean ± S.D. of triplicate in each group.
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