STUDY OF THE INHIBITORY EFFECTS OF VITAMIN E DERIVATIVES ON MITOCHONDRIAL COMPLEX II SUBUNIT USING MOLECULAR DOCKING

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ABSTRACT

Objective: The goal of this study was to create vitamin E derivatives and explore their potential anticancer properties using a computational approach.

Methods: The Steglich method was used for the synthesis of the vitamin E-fatty acid (pentaenoic acid, heptanoic acid, and octanoic acid) derivatives, with N,N'-dicyclohexyl carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as the catalysts. The structure of the synthesized products was determined by ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and liquid chromatography-mass spectrometry (LC-MS). Molecular docking was carried out on the succinate dehydrogenase (SDH) enzyme using AutoDockTools.

Results: α-Tocopheryl pentanoate (α-TP), α-tocopherol heptanoate (α-TH), and α-tocopherol octanoate (α-TO) were the three vitamin E derivatives synthesized in this study. Based on the results of molecular docking, the novel compounds (α-TP, α-TH, and α-TO) generated bond energies of −10.57, −9.61, and −9.20 kcal/mol, respectively.

Conclusion: All newly synthesized compounds exhibited lower binding affinity values than α-tocopherol (α-T). This confirms that these compounds might not provide greater advantages than α-tocopherol in terms of inhibitory effects on mitochondrial complex II (CII).

Keywords: Vitamin E, Pentanoic acid, Heptanoic acid, Octanoic Acid, SDH

INTRODUCTION

Succinate dehydrogenase (SDH), also known as mitochondria complex II or succinate-ubiquinone oxidoreductase, is a mitochondrial enzyme that plays a role in both the citric acid cycle and the electron transport chain (ETC) across all living organisms [1]. This enzyme catalyzes the oxidation of succinate to fumarate and the reduction of ubiquinone (UbQ) to ubiquinol [2]. SDH comprises SDHA, SDHB, SDHC, SdhD, SDHAF1, SDHAF2 subunits [3], and exhibits tumor-suppressing effects [4]. A decreased SDH activity leads to the accumulation of succinate, thus triggering the stabilization of the hypoxia-inducible factor (HIF) through competitive inhibition of HIF prolyl hydroxylases [5]. A stabilized HIF triggers pseudo-hypoxia signaling, leading to angiogenesis, dysregulation of cell proliferation, and adhesion [6]. Accumulation of succinate is also linked to epigenetic changes that promote oncogenesis by inhibiting histone demethylation [7]. The decreased in SDH activity can arise from mutations in SDHA, SDHB, SDHC, SdhD, or SDHAF2 (SDHx genes) [8] and has been documented in gastrointestinal stromal tumor (GIST) [9], renal cell carcinoma (RCC) [10], pituitary adenoma (PA) [11], and pancreatic neuroendocrine tumor (PanNET) [12].

Foods and medicinal plants represent important resources for the discovery of novel and valuable therapeutic molecules [13]. Biomolecules exhibit remarkable diversity and possess potent antioxidant, anti-inflammatory, and anticancer properties [14]. Quantitatively, structure-activity relationship studies have indicated that biomolecules can serve as templates for chemical modifications to enhance the efficiency, safety, and bioavailability of compounds [13]. α-Tocopherol (α-T), a natural substance found in fats and oils from both animal and vegetable sources, is among these biomolecules [15]. Recent research has revealed the diverse biological functions of α-T, encompassing anti-inflammatory [16], anticancer, and antioxidant properties [17]. Extensive research on α-T has been conducted to modify its structure to generate vitamin E derivatives with antioxidant and anticancer properties [18]. The synthesis of α-tocopherol succinate (α-TS), a derivative of vitamin E, was accomplished through the combination of α-T and succinic acid [19]. In mouse models of breast cancer, α-TS substantially inhibited tumor progression [20]. This was achieved by α-TS blocking the UbQ binding sites of CII, leading to the generation of reactive oxygen species (ROS) and the subsequent induction of apoptosis [21]. α-TS acts dominantly downstream of any anti-apoptotic pro-survival activity resulting from erbB2 receptor tyrosine kinase signaling [22]. Meanwhile, α-tocopherol acetate (α-TA) was synthesized through the chemical acylation of α-T, using acetic acid or acetic anhydride as acyl donors and a metal catalyst [23]. Previous research has documented the in vivo potency of α-TA in suppressing the metastasis of lung cancer cells [24] and triggering apoptosis in breast cancer cells (MCF-7) [25]. The conceptual framework for modifying the structure of α-T through its synthesis with fatty acids (e.g., pentaenoic acid (PA), heptanoic acid (HA), and octanoic acid (OA)) aims to obtain potent derivatives of α-T. The synthesized compounds, specifically α-tocopherol pentanoate (α-TP), α-tocopherol heptanoate (α-TH), and α-tocopherol octanoate (α-TO), are expected to demonstrate antioxidant and anticancer activities. According to the (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the IC₅₀ values for PA on MDA-MB-231/RB breast cancer cells were 7.87±1.2 mmol after 48 h of incubation [26] and 4.88±5.4 mmol after 72 h of incubation [27]. Meanwhile, the IC₅₀ values for heptanoic acid (HA) on HepG2 liver cancer cells were 1.73±0.6 mmol after 48 h of incubation [28] and 0.89±0.6 mmol after 72 h of incubation [29]. OA enhanced the activity of caspase 8 [30] and cyclin-dependent kinase inhibitor 1 (P21) in all three cell types [31,32]. Studies on the synthesis and activity of α-TP, α-TH, and α-TO are lacking. Hence, we synthesized α-TP, α-TH, and α-TO through the Steglich esterification method [32], and explored the possibility of their inhibitory effects on CII in silico.

MATERIALS AND METHODS

Materials

Organic solvents and chemicals were analytical grade and were purchased from Sigma Aldrich Co. (India, Surabaya, Indonesia): n-hexane, chloroform, ethyl acetate, methanol, dimethyl sulfoxide
The structures of the SDH of Escherichia coli (SQR) is closely analogous to CI [33]. The redox center of SQR plays a role in preventing the production of ROS on the flavin adenine dinucleotide (FAD) [34]. Hence, SQR is expressed during aerobic respiration to neutralize the activity of the fumarate reductase enzyme, known for its high production of ROS [34]. The SDH structure was acquired from the Protein Data Bank (PDB, http://www.rcsb.org) with the code 1NEK and had a resolution of 2.60 Å. A Hewlett-Packard notebook (2018) featuring an Intel(R) Core(TM) i5-8250U CPU @ 1.60GHz, 8 GB RAM, and a Radeon 530 graphics card, and preloaded with the Windows 11 22H2 operating system was used to process the data. ChemDraw 181 and Chem3D 181 (PerkinElmer Informatics), AutoDock4.2 Tools Version 1.5.7, and BIOVIA Discovery Studio 21.1.0.0 (Discovery Studio 2021 Client, Dassault Systèmes) were used.

Ligand and protein preparation for in silico molecular docking

The macromolecular complex (1NEK) was separated from water solvent molecules and natural ligands. The macromolecule was optimized using AutoDock4.2 Tools Version 1.5.7, including the addition of polar hydrogens and Kollman charges. Finally, the file was saved in pdbqt format (.pdbqt) [35]. The natural ligand (FAD, C=H=NO+P), used as a reference, was obtained from the PDB (http://www.rcsb.org). Meanwhile, the test ligands (α-TP, α-TH, α-T0) were drawn, saved in cdx format (.cdx), and converted to pdb format using Chem3D 10.1 [36]. Both ligands were subjected to optimization with AutoDock4.2 Tools Version 1.5.7, incorporating a setting for adding hydrogen, charges, and a torsion tree. Afterward, the files were saved in pdbqt format (Protein Data Bank, partial charge (q), and atom type (t)) [37].

Redocking simulation with AutoDock4.2 tools

A grid box defines the spatial constraints for ligand docking on the macromolecule [38], set at coordinates (x = 80.925 Å; y = 88.698 Å; z = 146.511 Å) with a box volume of 60 Å × 42 Å × 40 Å. This was then saved in gpf format (grid parameter file). The next step involves selecting parameters using the Lamarckian GA method, to be applied in running AutoDock4.2 Tools Version 1.5.7, which was saved in dpf format (docking parameter file). The parameters resulting from the molecular docking were analyzed using AutoDock4.2 Tools. The docking parameters, including binding free energy (ΔG) values, inhibition constants (Ki), and root mean square deviation (RMSD), can be observed in the dgl file (docking log file) using the Notepad application. The interactions between ligands and the amino acid residues of the enzyme were visualized using BIOVIA Discovery Studio visualizer 21.1.0.0 [38].

RESULTS

Synthesis of the compounds (α-TP, α-TH, α-T0)

Vitamin E derivatives were synthesized, and their anticancer activity was evaluated through molecular docking. In this study, the Steglich method [18], which involves esterification with DCC and DMAP catalysts, was used to synthesize three vitamin E derivatives. Steglich esterification typically occurs at room temperature, but in this case, the reaction was carried out at 50°C. Elevating the temperature increases the reaction rate. Fig. 1 illustrates the reaction steps for the synthesis of the compounds. The α-TP product was obtained in a yield of 60.66%, resulting in 1.5272 g of yellowish oil with an RF value of 0.63 in 9% ethyl acetate in n-hexane. The pure α-TH exhibited a light-yellow color, with a yield of 58.90% (1.2281 g) and an RF value of 0.70 in 9% ethyl acetate in n-hexane. The pale-yellow oil of pure α-T0 was 1.1232 g (58.20%, RF = 0.65 in 9% ethyl acetate in n-hexane).

Structural analysis of the compounds (α-TP, α-TH, α-T0)

The UV spectral data showing the chromophore group and the maximum absorbance of compounds are presented in Table 1. All compounds exhibited the maximum UV absorption at a wavelength of 204 nm, indicating the n → π* electronic transition of the carbonyl (C=O) chromophore group in the ester. Thus, the three synthesized compounds belong to the ester type.
The results of the IR spectrum analysis indicate that all the generated compounds are esters with aliphatic CH groups (wavenumber of 2864−2924 cm⁻¹), carbonyl (C=O) groups in esters (wavenumber of 1754−1755 cm⁻¹), and C–O groups in ethers (wavenumber of 1458 cm⁻¹). The LC-MS spectrum indicated peak ions for α−TP (M+H) at 514.26, 515.26, 556.67 g/mol, and C−O groups in ethers (wavenumber of 1458 cm⁻¹). The results of the IR spectrum indicate peak ions for α−TP (M+H) at 514.26, 515.26, 556.67 g/mol, and α−TH are 514.26, 557.67 g/mol, respectively. This suggests that the actual molecular weights of α−TP, α−TH, and α−TO are 515.26, 542,889, and 556.67 g/mol, respectively. These molecular weights align with the calculations made using ChemDraw 18.1.

Redocking simulation and molecular docking with AutoDock4.2

The RMSD values indicate the difference in the positions of ligand atoms compared to their natural state before the docking process [39]. A smaller RMSD value indicates minimal alteration to the natural state of the ligand. An acceptable RMSD deviation is typically less than 2.5 Å. The validation results of the natural ligands against the protein resulted in an RMSD of 2.11 Å, indicating the use of valid docking parameters. Hence, the docking method can be employed for the test ligands.

The results of molecular docking between the compounds with 1NEK are presented in table 2 and fig. 2. Molecular docking provides information on binding free energy (∆G), inhibition constants (Ki), and the interaction between compounds and the protein.
The compounds in this study were synthesized through the combination of α-T and fatty acids (PA, HA, and OA). The OH alcohol group in α-T acts as a feeble nucleophile, leading to a slow reaction rate when attacking the less reactive carbonyl carbon in fatty acids. Thus, a catalyst is necessary for the esterification reaction between α-T and fatty acids. Here, DCC and DMAP were used as catalyst in the Steglich esterification [32]. While this reaction typically takes place at room temperature, higher temperatures are employed in this study to accelerate the reaction. Chloroform serves as the inert solvent, and vitamin E exhibits high solubility in chloroform. The nitrogen atom of DCC has free electron pairs that act as a weak nucleophile, leading to a slow reaction rate when attacking the less reactive carbonyl carbon in fatty acids. Thus, a catalyst is necessary for the esterification reaction between α-T and fatty acids. Here, DCC and DMAP were used as catalyst in the Steglich esterification [32]. While this reaction typically takes place at room temperature, higher temperatures are employed in this study to accelerate the reaction. Chloroform serves as the inert solvent, and vitamin E exhibits high solubility in chloroform.

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The nucleophilic nature of fatty acids arises from the removal of hydrogen atoms, leading to the presence of negatively charged oxygen. Isoourea possesses a carbon center deficient in electrons, rendering it highly prone to attack by negatively charged nucleophiles, resulting in the formation of a reactive acyl intermediate known as O-acylisourea. DMAP serves as an acyl transfer agent, initiating an attack on O-acylisourea to generate a reactive amide intermediate (an active ester) [41]. This intermediate subsequently undergoes a swift reaction with α-T, resulting in the formation of α-tocopherol carboxylate with an ester bond.

The ongoing challenge in cancer treatment is primarily attributed to persistent mutations, rendering tumor cells resistant to conventional chemotherapeutic agents [42]. A contributing factor to the elevated incidence of cancer, its metastatic capability, and frequent resistance to treatment is the existence of tumor-initiating cells (TICs) [43]. These cells make up a small subset of the tumor, possessing the ability to self-renew and facilitate tumor growth [44]. Recent studies have shown the crucial role of TICs in the initiation and progression of tumors [45]. Consequently, targeted therapies aimed at TICs could impede tumor [46] growth and potentially eliminate the pathology [46]. The exploration of anti-TIC approaches has thus become a research topic of interest. Mitocans, a class of compounds that have demonstrated anticancer properties through the destabilization of mitochondria, seems to exhibit efficacy against TICs [21]. Mitocans are small molecules that induce apoptosis in cancer cells by targeting mitochondria [21]; they are categorized into various groups based on their molecular target. α-TS is an example of mitocan [47], which affects CI by disrupting the function of UbQ to self-aggregate, leading to the production of ROS, in turn initiating selective apoptosis in cancer cells [47].
Our results showed a relationship between anticancer properties and the modification α-T by introducing an additional carboxylic substituent (e.g., pentanoate or heptanoate or octanoate) at the C-6 position. The C-H groups of the novel compounds were tightly bonded to the active site of SDH by several van der Waals, hydrophobic, and electrostatic interactions. TM, after O=S, conventional hydrogen bonds seemed to have a minor impact on the affinity of the novel compounds with the SDH-like protein (fig. 2). Molecular docking of CII revealed that the free binding energies were in the ascending order of α-Tα-α-Tα=α-Tα=α-Tα=α-Σα=α-Σα=TA-, with respective values of -9.20,-9.61,-10.57,-10.80,-10.87, and -11.21 kcal/mol. Nonetheless, further studies in vitro and in vivo targeting CII are required to elucidate the anticancer activity of the synthesized compounds and validate their suitability as potential drug candidates. The outcomes of enzymatic and cytotoxic assays may differ from the in silico docking results and could provide new insights into the mechanism of action of the novel compounds.

CONCLUSION

The vitamin E derivatives α-tocopherol pentanoate (α-TP), α-tocopherol heptanoate (α-TH), and α-tocopherol octanoate (α-TO) were synthesized using the Steglich esterification method. All the synthesized compounds exhibited low binding affinity, demonstrating their lack of efficacy as potential cancer drug candidates. However, further research is needed to validate the inhibitory effects of the novel compounds in vitro and in vivo on mitochondrial CII in the most common types of cancer worldwide.

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AUTHORS CONTRIBUTIONS

Irma Ratna Kartika: Conceptualization, Investigation, Methodology, Writing-original draft. Teni Erawati: Conceptualization, Investigation, Methodology. Sri Widia A. Jusman: Conceptualization, Investigation, Methodology. Mohamad Sadikin: Conceptualization, Investigation, Methodology.

CONFLICT OF INTERESTS

All authors have no conflicts of interest.

REFERENCES


