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Study of Antioxidant Activity of the Derivatives of Quinoline-4-carboxylic Acids by the Modification of Isatin via Pfitzinger Reaction

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Abstract

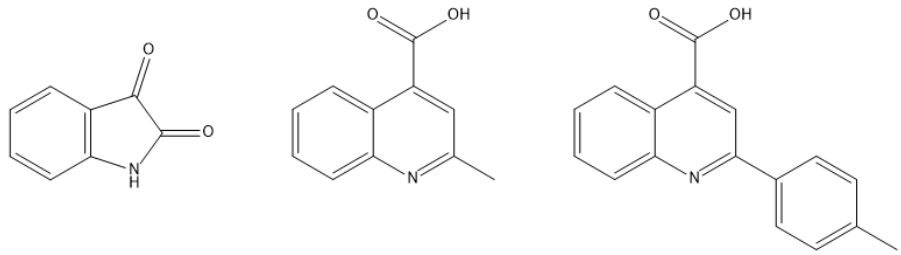
In this study, a quinoline-4-carboxylic acid derivative was synthesized through Pfitzinger reaction. In this reaction, isatin is modified via its reaction with ketone and refluxed for 24 h to obtain quinoline-4-carboxylic acid. The presence of a carboxylic group was identified by Fourier Transform Infrared (FTIR) spectroscopy and ultraviolet–visible (UV-Vis) spectrophotometry. The results showed that the absorption peaks of C=O and O–H stretching's were detected in the range of 1724–1708 and 3436–3242 cm^{-1}, respectively. In the UV-vis spectrum, a shift in the absorption peak was observed toward a larger wavelength, which is referred as a bathochromic shift. The formation of quinoline-4-carboxylic acid derivative was also characterized using the mass spectrometry method. The modification of isatin aims to increase antioxidant activity to obtain quinoline-4-carboxylic acid, which has a better inhibition percentage than isatin. Antioxidant tests were conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The antioxidant activity is measured based on the ability of quinoline-4-carboxylic acid derivative to donate hydrogen radicals, which revealed that the product had a relatively better inhibitory effect than isatin. At a concentration of 5 mg/L, isatin did not show antioxidant activity with the DPPH method. By contrast, the inhibition percentages of 2-methylquinoline-4-carboxylic acid and 2-(4-methylphenyl)quinoline-4-carboxylic acid were approximately 30.25% and 40.43%, respectively. Furthermore, the presence of an aromatic ring makes the antioxidant activity.

Keywords: antioxidant, isatin, Pfitzinger reaction, quinoline-4-carboxylic acid, quinoline derivatives

Introduction

Quinoline, a heterocyclic compound composed of benzene and pyridine rings, is found in many secondary metabolites. Based on the structure, the two constituents of quinoline can undergo different reactions. Because of its stability and ease of preparation, quinoline is widely used as a precursor in the synthesis of derivative compounds and natural compounds [1]. The commonly used quinoline framework as a key structure in the modification of a compound is quinoline-4-carboxylic acid. The carboxylic group part of the compound allows it to be modified with natural compounds so that the quinoline-4-carboxylic acid compound becomes a key structure in the synthesis of quinoline derivatives [2–5].

Quinoline-4-carboxylic acid compounds can be synthesized through various methods, such as the Pfitzinger method, the reaction between isatin and ketone with a potassium hydroxide catalyst [6]; Doebner–von Miller method, the reaction between aniline, aldehyde, and pyruvic acid [7]; Conrad–Limpach method, the condensation reaction between aniline and β-keto ester [8]; and Combe method, the reaction between aryl amines and β-diketones [9]. Compared with other methods, the Pfitzinger method can synthesize quinoline-4-carboxylic acid with relatively simple and rarely produces by-products. This method can also be used for various substituents, that is, both aliphatic groups and carboxylic derivatives [10]. In this method, a mixture of isatin and ketone is heated under a reflux process to obtain quinoline-4-carboxylic acid [11–13]. Isatin is used because it has a structure that is easy to modify into quinoline derivatives. Isatin is a heterocyclic amine compound with a six-member benzene ring and a five-member ring with two adjacent carbonyl groups. Under strong alkaline conditions, isatin will first experience ring opening and then undergo cyclization through a heating process with reflux [14]. Therefore, isatin is widely used as a precursor in the synthesis of quinoline derivatives. In several studies, isatin-modified compounds exhibit antibacterial [1], antiviral [15], antioxidant [16], and anticancer [17] activities. In this experiment, aliphatic ketones (i.e., acetone) and aromatic ketones (i.e., 4-methyl acetophenone) were used to show the effect of the aromatic ring of quinoline acid derivatives on the inhibition ability of the compounds.



(a) (b) (c)

Figure 1. Structures of the (a) Isatin and Quinoline-4-Carboxylic Acid Derivatives: (b) 2-Methylquinoline-4-Carboxylic Acid and (c) 2-(4-Methylphenyl)Quinoline-4-Carboxylic Acid

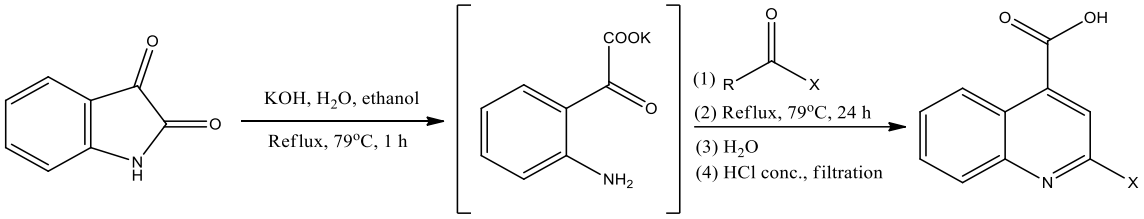


Figure 2. Scheme of the Pfitzinger Reaction in this Study (X = Methyl or 4-Methylphenyl)

The structures of isatin and quinoline-4-carboxylic acid derivatives are represented in Figure 1. As outlined in Figure 2, isatin was synthesized by Pfitzinger method into the quinoline-4-carboxylic acid derivatives.

Antioxidants are molecules that can inhibit oxidation reactions caused by free radicals. Several mechanisms of inhibition of free radicals include hydrogen donation, singlet oxygen radical quenching, and metal ion chelation [18]. There are several assays of testing antioxidant activity in vitro, including the DPPH (2,2-diphenyl-1-picrylhydrazyl), NBT (nitro blue tetrazolium), FRAP (ferric reducing-antioxidant power), TRAP (total reactive oxygen potential), TAR (total antioxidant reactivity), and others. All the antioxidant assays are complementary because the parameters measured are different. The DPPH assay is the simplest antioxidant assay because it is fast, relatively inexpensive and does not require many reagents and reaction steps [19]. The modification of isatin to quinoline-4-carboxylic acid is expected to increase the antioxidant activity of these compounds.

Methods

Synthesis of quinoline-4-carboxylic acids. This process used the method employed in Ref. [6] with some modifications. First, 0.02 mol KOH was dissolved in 1 mL water and 40 mL absolute ethanol in a round-bottom flask. Then, 0.0075 mol isatin was added and stirred continuously for 1 h at room temperature until the color changed from purple to brown. Subsequently, 0.015 mol ketone was gradually added to the solution. The resulting mixture was refluxed at 79°C while stirring for approximately 24 h in a heating mantle. The reaction was observed by thin-layer chromatography with the eluent *n*-hexane : ethyl acetate = 1 : 2. Next, 20 mL distilled water was added. Finally, the resulting solution was cooled and acidified by adding drops of concentrated hydrochloric acid. The precipitate formed was filtered and washed with 5 mL water (twice), 1 mL ethanol (twice), and 2 mL diethyl ether (twice). The resulting compounds were characterized using mass spectrometry, Fourier Transform Infrared (FTIR) spectroscopy, and ultraviolet–visible (UV-Vis) spectrophotometry.

Antioxidant with DPPH assay. Each of the samples was made with five concentration variants in the range of 1–5 mg/L with absolute ethanol as solvent. The DPPH solution was prepared by dissolving 0.4 mg DPPH in 100 mL ethanol. A 2 mL sample was added to 2 mL DPPH, and the solution was incubated for 30 min in the dark. Thereafter, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm for three repetitions, and the inhibition percentage was calculated using the following formula, where the negative control (blank) was ethanol and the positive control was ascorbic acid.

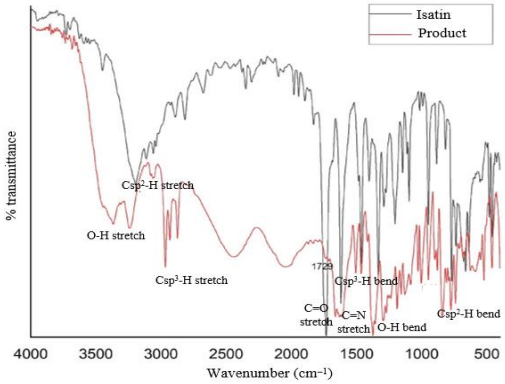
$$\% \text{ inhibition} = \frac{\text{abs blanc - abs sample}}{\text{abs sample}} \times 100\% \qquad (1)$$

Results and Discussion

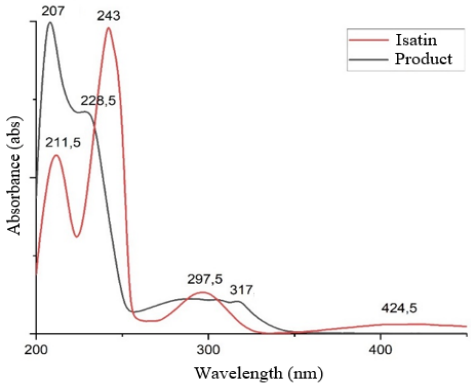
2-methylquinoline-4-carboxylic acid. **2-Methylquinoline-4-carboxylic acid.** Brown powder. $C_{11}H_9NO_2$. Exact mass = 187.0633. Yield = 67.30%. TLC with eluent of *n*-

hexane : ethyl acetate = 1 : 2 (Rf = 0.11). The FTIR spectrum of the product are represented in Figure 3a. At 3242 cm^{-1}, there is an absorption peak of the stretching vibration of the carboxylate O-H bond. The bending vibration absorption peak of the O–H carboxylate bond is detected at 1410 cm^{-1}. For the C=O carboxylic bond, the stretching vibration absorption peak is detected at 1724 cm^{-1}. The absorption peak of the stretching vibration of the C=N bond is detected at 1667 cm^{-1}. For the C sp^2–H aromatic bond, a stretching vibration absorption peak is observed at a wavenumber of 3068 cm^{-1} and the bending vibration absorption peak is observed at a wavenumber of 947 cm^{-1}. This product has a methyl substituent on the quinoline framework; thus, the stretching vibration absorption peak of C sp^3–H is observed at 2975–2870 cm^{-1} and the bending vibration absorption peak is observed at a wavenumber of 1508 cm^{-1}. The UV-vis spectrum analysis of isatin showed that it has three absorption peaks, namely at 211.5 nm (transition n → π* of the amide group), 243 nm (transition π → π* of the C=C group), and 297.5 and 424.5 nm (transition n → π* of the α-diketone group). The UV-Vis spectrum of this product (Figure 3b) showed absorption peaks at 207 nm (transition n → π* of the carboxylic group), 228.5 nm (transition π → π* of the C=N group) and 317 nm (transition π → π* of the C=C group). The absorption peak for α-diketones is no longer visible in the product spectrum and is replaced by an increase in the C=C bond. Based on this comparison, 2-methylquinoline-4-carboxylic acid experienced a shift in the absorption peak shift towards a larger wavelength or a bathochromic shift. Gas chromatography-mass spectrometry (GCMS) analysis identified the product with a relative molecular mass of 187.1 at a retention time of 13.462 (Figure 3c). This finding confirms the formation of the compound, 2-methylquinoline-4-carboxylic acid.

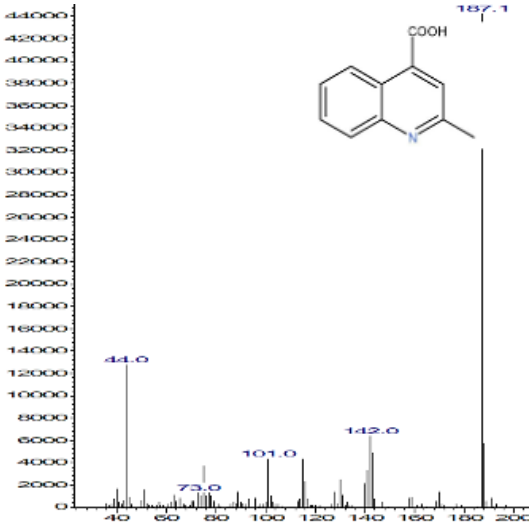
2-(4-Methylphenyl)quinoline-4-carboxylic acid. Light brown powder. $C_{17}H_{13}NO_2$. Exact mass = 263.0946. Yield = 79.93%. For substituted aromatic ketones, the results showed a better yield than the synthesis using substituted isatin as the precursor [20]. TLC with *n*-hexane : ethyl acetate eluent = 1 : 2 (Rf = 0.36). The FTIR spectrum depicts (Figure 4a) the stretching vibration absorption peaks of the O–H carboxylic bond, C sp^2–H aromatic bond, C sp^3–H aliphatic bond, C=O carboxylic bond, and C=N bond were detected at wavenumbers of 3436 cm^{-1}, 3037 cm^{-1}, 2968–2850 cm^{-1}, 1708 cm^{-1}, and 1605 cm^{-1}, respectively. Meanwhile, the bending vibration absorption peaks were detected at wavenumbers of 1453 cm^{-1} and 896 cm^{-1} for carboxylic O–H bond and aromatic C sp^2–H bond, respectively. The UV–Vis spectrum of this product (Figure 4b) showed absorption peaks at 207.5 nm (transition n → π* of the carboxylic group), 260.5 nm (transition π → π* of the C=N group), and 330 nm (transition π → π* of the C=C group). Similar to 2-methylquinoline-4-carboxylic acid, 2-(4-methylphenyl)quinoline-4-carboxylic acid experienced a shift in the absorption peak toward a larger wavelength. When comparing the UV–Vis spectrum of 2-(4-methylphenyl) quinoline-4-carboxylic acid and 2-methylquinoline-4-carboxylic acid, for the same transition,



(a)



(b)



(c)

Figure 3. (a) Fourier Transform Infrared (FTIR) Spectrum, (b) Ultraviolet–Visible (UV-Vis) Spectrum, and (c) Gas Chromatography-Mass Spectrometry (GCMS) Chromatogram for 2-Methylquinoline-4-Carboxylic Acid

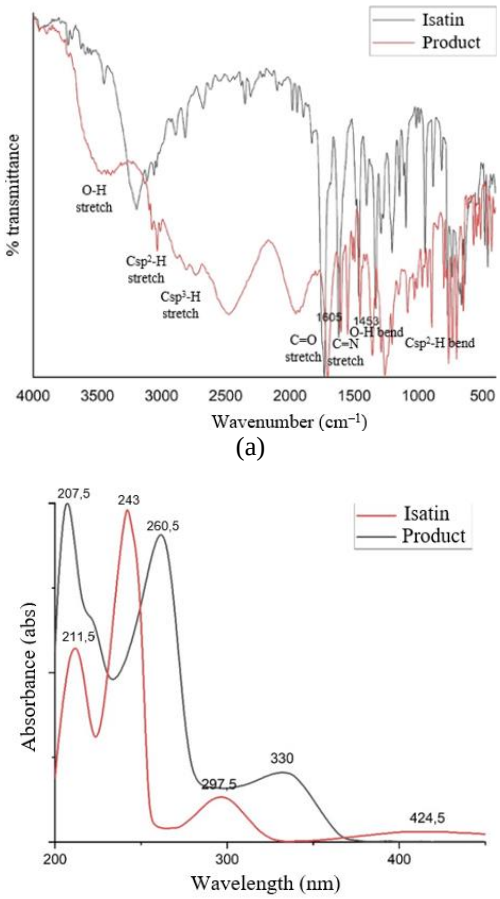


Figure 4. (a) FTIR Spectrum and (b) UV–Vis Spectrum of 2-(4-Methylphenyl)Quinoline-4-Carboxylic Acid

the absorption peaks of 2-(4-methylphenyl)quinoline-4-carboxylic acid occur at larger wavelengths because the substituent on 2-(4-methylphenyl)quinoline-4-carboxylic acid is a 4-methylphenyl group such that the transition $\pi \rightarrow \pi^*$ of the C=C group becomes longer.

Antioxidant with DPPH assay. Antioxidant data were obtained via testing using the DPPH method. DPPH is a relatively stable free radical that can accept electrons or hydrogen radicals; thus, it can be used as an indicator of antioxidant activity based on the ability of products to donate hydrogen atoms [21]. The test begins with finding the right concentration range until a color change from purple to yellow at low to high concentrations can be observed (Figure 5). The assay showed that both products have a relatively good inhibition percentage compared with isatin. At a concentration of 5 mg/L, the inhibition percentage of 2-methylquinoline-4-carboxylic acid was 30.25%, whereas that of 2-(4-methylphenyl)quinoline-4-carboxylic acid had a percent inhibition of 40.43%. The comparison of inhibition percentage between isatin and the products is shown in Figure 6. The antioxidant activity of the two quinoline-4-carboxylates can be derived

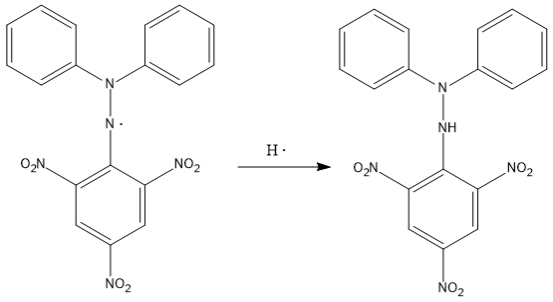


Figure 5. Radical Form (Purple) and Nonradical Form After Taking the Hydrogen (Yellow) of DPPH

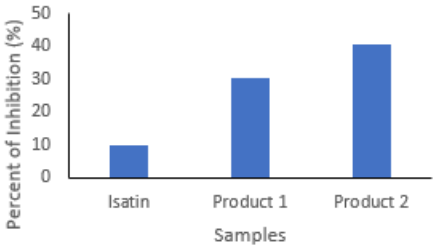


Figure 6. Inhibition Percentage at 5 mg/L Concentration. Product 1 is 2-Methylquinoline-4-Carboxylic Acid, and Product 2 is 2-(4-Methylphenyl)Quinoline-4-Carboxylic Acid

from the acidic H of the compound, which is relatively easy to release compared with isatin [22]. 2-(4-Methylphenyl)quinoline-4-carboxylic acid showed a better inhibition percentage than 2-methylquinoline-4-carboxylic acid; the inhibition percentages of 2-methyl quinoline-4-carboxylic acid and 2-(4-methylphenyl) quinoline-4-carboxylic were approximately 30.25% and 40.43%, respectively. This finding can be attributed to the fact that these compounds are more capable of stabilizing the quinoline radicals formed by resonance. Thus, isatin-modified compounds have better antioxidant activity than isatin.

Conclusion

This research succeeded in modifying isatin to produce two quinoline-4-carboxylic acid compounds. Based on the characterization, the two desired products have been formed successfully. The resulting compound showed an increase in antioxidant inhibition percentage compared with the antioxidant capacity of isatin. Products with aromatic rings showed better inhibition percentages. The products can be further modified to produce compounds with even better bioactivity.

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