Efficiency and potential synthesis of pyrimido [4,5-d] pyrimidine derivatives by iodine catalyst as antioxidant agent

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Abstract. Pyrimido [4,5-d] pyrimidine is an uracil compound that has similarities to the structure of pteridine and purines and has significant bioactivity. The aim of this research is to synthesize pyrimido [4,5-d] pyrimidine derivatives and test their activity as antioxidant. The reaction was carried out with the support of the iodine catalyst to get an effective reaction. Experimental evidence in support of the formation products was confirmed using FTIR, UV-vis Spectrophotometer, and GC-MS. The results of the analysis produced four pyrimido [4,5-d] pyrimidine derivatives, 5-Styryl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 1), 5-Styryl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 2), 5-Phenyl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 3), and 5-Phenyl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 4). Reaction optimization was conducted by varying reaction time, reaction temperature, amount of catalyst and solvent. The optimum conditions of the reaction were obtained under conditions of 10% mol of catalyst, temperature 70 °C, for 4 h with a water solvent. Compound 1 has a better activity than 3 other compounds with an IC50 value of 10.11 ppm.

Keywords: pyrimido [4,5-d] pyrimidine derivatives, iodine catalyst, cinnamaldehyde, barbituric acid

1. Introduction
Uracil derivatives containing nitrogen groups in their heterocyclic make it to have an attractive bioactivity ability. One of the significant uracil annulated is pyrimido[4,5-d]pyrimidine because it is similar to purine and pteridine system [1, 2]. Several pyrimido [4,5-d] pyrimidine derivative compounds have been reported to have a diverse bioactivity. Among them are hepatoprotective, antitumor, antimicrobial, anti-allergic effects, antioxidants, antivirus, antiallergic, antifungal, antihypertensive, anticancer, vasodilators, and cardiotonics [3-5].

The formation of pyrimido compounds [4,5-d] pyrimidine is obtained by the Biginelli reaction [6]. Previous research has done it by reacting aromatic aldehyde with barbituric acid and urea/thiourea [7, 8]. In this research, cinnamaldehyde is used as an alternative to aromatic aldehydes which will enrich the pyrimido [4,5-d] pyrimidine compounds. Cinnamaldehyde is an active compound found in cinnamon having bioactivity as an antibacterial, antifungal, antidiabetic, antiviral, anticancer, anti-inflammatory, and antioxidant [9, 10]. In this study, the reaction is supported by iodine catalyst. The application of
molecular iodine has gained much attention as a cheap, easy-to-obtain catalyst for various organic transformations. This catalyst has also been exploited for various types.

2. Materials and method

2.1. Chemical reagents and instrumentations
All materials and reagents were obtained from the company of Merck, Fluka, and Sigma-Aldrich and used without further purification. Merck grade Thin Layer Chromatography (TLC) plate was employed for thin layer chromatography to evaluate the progress of the reaction, visualized by UV light. The UV–Vis spectra was registered by Agilent 8453 spectrophotometer, Fourier transform infrared spectroscopy (FTIR) spectra was registered in the 4000–400 cm⁻¹ region, on a Shimadzu infrared spectrophotometer in KBr discs and Mass spectra (MS) was performed by GC-MS (Agilent).

2.2. General procedure for the synthesis of pyrimido [4,5-d] pyrimidine
The synthesis of pyrimido [4,5-d] pyrimidine was carried out by mixing barbituric acid (1 mmol), aldehyde (1 mmol), urea/thiourea (1 mmol), and iodine 10% mol. Then, the mixture was heated in distilled water at variation temperature under reflux conditions. The reaction was monitored and evaluated with TLC. After completion, the product was washed with ethanol, crystallized, and purified by hot ethanol. Then, the products were characterized for its absorbance, functional group, and molecular weight using UV/Visible Spectrophotometry, FTIR, and GCMS. Simple illustration of the reaction that occurred in this study is described in figure 1.

2.3. Antioxidant activity test
The antioxidant activity test used 1,1-diphenyl-2-picryl hydrazyl (DPPH) and the sample solution was prepared in a series of concentrations ranging from 5–1000 ppm in a methanol solvent. Absorbance of the control solution was measured for DPPH solution using methanol as a blank. A total of 2 mL of each solution was reacted with 1 mL DPPH in a tube. The reaction mixture was then incubated at room temperature for 30 min. After that, the absorbance of the solution was measured at a wavelength of 517 nm using a UV-vis spectrophotometer. The calculation of % free radical DPPH follows the following equation:

\[
\% \text{Inhibition} = \left( \frac{Ac - As}{Ac} \right) \times 100 \%
\]

where, \( Ac \) is the absorbance of DPPH control, and \( As \) is the absorbance of sample. IC₅₀ values were determined using a linear regression correlation between concentration and % free radical DPPH. IC₅₀ is a concentration value that scavenging 50 % of DPHH free radicals [11, 12].

3. Results and discussion
The synthesis of pyrimido [4,5-d] pyrimidine-derived compounds using iodine as a catalyst has been synthesized through a one-pot three-component method. Analysis of the maximum wavelength formed, compound 1 was measured with a Uv-vis spectrophotometer and obtained a maximum wavelength at 246 nm and 377 nm (figure 2a). The rightward shift is caused by additional substituents and called as a bathochromic shift or redshift. Experimental evidence in support of the formation pyrimido [4,5-d] pyrimidine compound is also obtained from the analysis of the functional groups of the target compound through the FTIR spectrophotometer. FTIR spectrum of compound 1 (figure 2b) shows several peaks that identify the vibrations of the functional group of this compound. A peak at wavenumber of 3170 cm⁻¹ is the stretching vibrations of secondary amines (N–H). In addition, there is also a bending vibration N-H at wave number 1571 cm⁻¹ with strong intensity. Besides that, the peak at 3083 cm⁻¹ is a
The stretching vibration of C–H aromatic (sp²) found on the benzene ring. While the peak at 2887 cm⁻¹ is stretching vibration C–H (sp³). The stretching vibration of the C–N is at 1420 cm⁻¹, C=C from aromatics at 1620 cm⁻¹ and C=O at 1756 cm⁻¹.

The main support in determining the structure of formation pyrimido [4,5-d] pyrimidine-derived compounds in this study is the use of GC-MS for the determination of the molecular weight of the target compound. Figure 3 shows the GC-MS spectrum of compound 1 with a parent peak m/z 284. Compound 1 which is a product of the reaction between cinnamaldehyde, barbituric acid, and urea would produce a compound with the molecular formula C₁₄H₁₂N₄O₃, with a molecular weight of 284 g/mol. The name of compound 1 is 5-Styryl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione. Some fragments formed by compound 1 are found at m/z 242, 199, 176.8, 155, and 127.

Evaluation of the ability of iodine as a catalyst in the synthesis reaction of pyrimido [4,5-d] pyrimidine derivatives was conducted by varying the temperature, reaction time, type of solvent, and iodine concentration as in table 1. From some of these experiments, it is known that the optimum conditions of the reaction are achieved at conditions of 10 % mol I₂, for 4 h at 70 °C with a water solvent. The yield obtained at this optimum condition is 65.45 %. Based on the optimum condition information in table 1, the reaction to enrich the derivative of compound pyrimido [4,5-d] pyrimidine was conducted by varying the aromatic aldehyde and replacing urea into thiourea. Data on the characteristics of pyrimido [4,5-d] pyrimidine-derived compounds can be seen in table 2.

**Figure 1.** Reaction scheme for the synthesis of pyrimido [4,5-d] pyrimidine derivatives.

**Figure 2.** (a) UV-vis and (b) FTIR spectra of compound 1.
Table 1. Optimization of the reaction condition for the synthesis of pyrimido[4,5-d]pyrimidine (compound 3).

| Entry | Catalyst | Time (h) | Temp. (°C) | Solvent | % yield |
|-------|----------|----------|------------|---------|---------|
| 1     | I₂ (10 mol%) | 2        | 100        | H₂O     | 48.74 % |
| 2     | I₂ (10 mol%) | 4        | 100        | H₂O     | 51.22 % |
| 3     | I₂ (10 mol%) | 5        | 100        | H₂O     | 51.80 % |
| 4     | I₂ (10 mol%) | 6        | 100        | H₂O     | 51.77 % |
| 5     | I₂ (10 mol%) | 8        | 100        | H₂O     | 41.64 % |
| 6     | I₂ (10 mol%) | 4        | 90         | H₂O     | 53.54 % |
| 7     | I₂ (10 mol%) | 4        | 80         | H₂O     | 56.54 % |
| 8     | I₂ (10 mol%) | 4        | 70         | H₂O     | 65.45 % |
| 9     | I₂ (5 mol%)  | 4        | 70         | H₂O     | 55.51 % |
| 10    | I₂ (15 mol%)| 4        | 70         | H₂O     | 63.45 % |
| 11    | I₂ (10 mol%)| 4        | 70         | Ethanol | 49.76 % |
| 12    | I₂ (10 mol%)| 4        | 70         | No solvent | 52.00 % |

Based on the structure formed, the name of these compounds are 5-Styryl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 1), 5-Styryl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 2), 5-Phenyl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 3), and 5-Phenyl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 4).

The antioxidant activity test was carried out using the DPPH method. Each sample solution was prepared with a concentration range of 5 to 1000 ppm in a methanol solvent. Each sample solution was added with 2 mL DPPH, then allowed to stand for 30 min in a dark room and absorbance of sample solution was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The antioxidant activity of compounds 1, 2, 3 and 4 was measured along with its precursor. Absorbance values obtained from measurements are used to get % inhibition. Table 3 shows the comparison of IC₅₀ of each compound. IC₅₀ is a concentration value which can capture 50 % of DPPH free radicals.
Table 2. Characterization data and yield of pyrimido [4,5-d] pyrimidine derivatives.

| No | Product | Characterization data | Yield (%) | Photo |
|----|---------|-----------------------|-----------|-------|
| 1  | ![Product 1](image1.png) | Yellow powder. IR (cm\(^{-1}\)): 3170 (N-H stretching), 1571 (N-H bending), 3083 (C-H sp\(^2\)), 2887 (C-H sp\(^3\)), 1420 (C-N), 1620 (C=C), and 1756 (C=O). UV-Vis (nm): 246 and 377. MS (m/z): 284 | 79.58 | ![Photo 1](image2.png) |
| 2  | ![Product 2](image3.png) | Yellow powder. IR (cm\(^{-1}\)): 3201 (N-H stretching), 1539 (N-H bending), 3077 (C-H sp\(^2\)), 2844 (C-H sp\(^3\)), 1416 (C-N), 1622 (C=C), and 1763 (C=O). UV-Vis (nm): 384. MS (m/z): 300 | 86.70 | ![Photo 2](image4.png) |
| 3  | ![Product 3](image5.png) | White powder. IR (cm\(^{-1}\)): 3236 (N-H stretching), 1521 (N-H bending), 3004 (C-H sp\(^2\)), 2890 (C-H sp\(^3\)), 1407 (C-N), 1625 (C=C), and 1725 (C=O). UV-Vis (nm): 248. MS (m/z): 259. | 69.48 | ![Photo 3](image6.png) |
| 4  | ![Product 4](image7.png) | Pale yellow powder. IR (cm\(^{-1}\)): 3173 (N-H stretching), 1500 (N-H bending), 3004 (C-H sp\(^2\)), 2866 (C-H sp\(^3\)), 1411 (C-N), 1618 (C=C), and 1724 (C=O). UV-Vis (nm): 262. MS (m/z): 275 | 65.45 | ![Photo 4](image8.png) |

Table 3. Antioxidant activity test of pyrimido [4,5-d] pyrimidine derivatives.

| Sample          | IC\(_{50}\) (ppm) |
|-----------------|-------------------|
| Compound 1      | 10.11             |
| Compound 2      | 32.74             |
| Compound 3      | 168.09            |
| Compound 4      | 109.11            |
| Benzaldehyde    | 797.22            |
| Cinnamaldehyde  | 579.32            |

Antioxidant activity (table 3) shows that compound 1 has a better activity than 3 other compounds. Its low IC\(_{50}\) value means that the compound can inhibit the free radicals by as much as 50%. The smaller the concentration needed to inhibit it, the better its ability as an antioxidant. Compound 1 was synthesized using cinnamaldehyde which also has antioxidant activity. However, when compared to its precursors, compound 1 has a significant increase in antioxidant activity.

4. Conclusion
The pyrimido [4,5-d] pyrimidine derivatives were successfully synthesized using cinnamaldehyde and benzaldehyde as a precursor through the one-pot three-component method with iodine catalyst. The characterization of synthesized compounds was evaluated with some instruments like FTIR,
UV-vis, and GC-MS and showed the characteristics of the target compound. The results identified that the compound formed was pyrimido [4,5-d] pyrimidine derivatives namely 5-Styryl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 1), 5-Styryl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 2), 5-Phenyl-5,8-dihydro-1H,6H-pyrimido[4,5-d]pyrimidine-2,4,7-trione (compound 3), and 5-Phenyl-7-thioxo-5,6,7,8-tetrahydro-1H-pyrimido[4,5-d]pyrimidine-2,4-dione (compound 4). The optimum condition reaction was carried out at 10 % mol I$_2$, for 4 h at 70 °C with a water solvent. Compound 1 has better activity than 3 other compounds with an IC$_{50}$ value of 10.11 ppm.

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