Naturally Abundance Vanillin as Starting Material to Synthesizing 4-(4-Hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one

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ABSTRACT

Indonesia is the second biggest producer of natural vanillin. Traditionally it was isolated from the bean of vanilla (Vanilla planifolia Andrews). This paper reports on applying vanillin as starting material for synthesizing a biologically important chemical structure 3,4-dihydropyrimidinone. The reaction was undertaken in one step following multi component reaction (MCR). Products determination was undergone using FTIR and UV-Vis spectrophotometry, and also liquid chromatography-mass spectrometry (LCMS). After purification under flash column chromatography in ethyl acetate-hexane, it was found a white solid of 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one in 67% yield with a few amount of an unreacted vanillin.

Key word: Vanilla planifonia, multi component reaction, dihydropyrimidinone

INTRODUCTION

Vanillin is an aldehyde compound, also known as 4-hydroxy-3-methoxybenzaldehyde. It was isolated naturally from the beans of vanilla plant (Vanilla planifonia A.) [1,2]. Indonesia has been becoming the second largest producer of natural vanillin after Madagascar [3,4]. The total domestic production was mostly sent to overseas mainly for US market. It was also reported about 37 metric ton per month of US vanillin import from Indonesia. This total import reached to 195 MT during January-November 2008 [5]. According to the Directorate General of Plantation, Department of Agriculture Indonesia, total area of vanilla plantation was 25,429 hectares until 2008 [6], and this has been growing widely until recent decades. However this increasing total production did not contribute significantly for improving a domestic income [7]. Thus diversifying the potency and application rather than direct export as raw material of the local natural vanillin to gain more values and economic benefits become an important strategy.

The interesting methodology that applies an aldehyde molecule as starting material to afford an important molecular backbone for medicinal and pharmaceuticals application is multicomponent reaction (MCR). It was defined as a reaction that involves more than two reactants to producing a specific single product contained essential part of the reactants in single step process [8]. Biginelli reaction is an example first developed by P. Biginelli (1893), involved three reagents to afford dihydropyrimidinone [9]. Beside an aldehyde, urea or thiourea and β-keto-ester was needed. Generally, reaction requires acidic catalysis in ethanol
under reflux stirring (Figure 1) [10]. Some examples of molecules have been prepared including evaluation of their biological activity such as antifungal, antibacterial [11,12,13,14,15,16,17,18], antiinflammation [11], and antitumor [18] have been reported recently. This paper discloses a recent application of vanillin, isolated from Indonesian natural source of Vanilla planifoli Andrews as starting material for synthesis dihydropyrimidinone alkaloid.

Figure 1. Schematic of Biginelli reaction

EXPERIMENT

Chemicals and instrumentation

Chemicals for this research were used as received from the manufacturer or as mentioned. Vanillin gave from local producer (98% purity), acetone (Merck), urea (Gresik Petrochemical Ltd.), magnesium sulfate anhydrate (Merck), ethyl acetate (Smart Lab, re-distillated), n-hexane (Smart Lab, re-distillated), ethanol (Smart Lab), pre-coated of TLC silica gel F254 (Merck), silica gel 60 (Merck).

Instrumentation operated for analysis such as gas chromatography-mass spectrometry (Shimadzu GCMS-QP2010S), infrared spectrophotometry (Shimadzu Shimadzu FTIR-8400S), UV-Vis Spectrophotometry (UV-Vis Shimadzu 1601), LCMS/MS (LC conditions; column xxxx, solvent 1.0% formic acid in methanol isocratic, pump pressure 10.0 bar using Accela 1250 pump type. While MS using TSQ MS with 9.0 min total run time, scan event 1, +c SIM Q1MS), analytical balance (Mettler Toledo).

Synthesis procedure

A dried of a 100 mL round-bottom flask was added vanillin (304.29 mg; 2.00 mmol), urea (120.11 g, 2.00 mmol), and acetone (2.00 mL, 27.24 mmol). A drop of glacial acetic acid was added. This mixture was stirred at 60 °C until reaction complete by monitoring in TLC. The product mixture was extracted with ethyl acetate, and dried under magnesium sulfate anhydrate. Then, the product was concentrated using rotary evaporator in vacuum. The crude product was further purified using flash column chromatography with silica as stationary phase and n-hexane/ethyl acetate as solvent. After TLC monitoring, the product fraction was added magnesium sulfate anhydrate and concentrated in vacuum to afford a pure white solid of 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one in 67% yield. Analysis using UV-Vis spectrophotometer found λmax in 214.60, 232.40, 284.20, 308.20, and 350.40 nm (solution in ethanol). FTIR (KBr plate, cm⁻¹) 3435.31, 3358.77, 3218.97, 1670.30, 1623.36; The GCMS analysis using Agilent developed method on Agilent manual for analysis alkaloid (injector temperature 200 °C, column temperature 210 °C isotherm for 30 min) did not give both of chromatogram and mass spectra data. LCMS/MS (Low resolution) found a single peak at 4.55 min with molecular weight 235.00 (100% intensity). Theoretical calculation for C₁₂H₁₄N₂O₃ is 234.10. ¹H-NMR (400 MHz, methanol-d₆) 2.26 (3H, s, CH₃), 3.83 (3H, s, OCH₃), 5.35 (1H, s overlap, OH), 5.36 (1H, d, H5), 5.56 (1H, d, H4), 5.83 (2H, s, broad, 2xNH), 6.80-7.25 (3H, m, ArH). ¹³C-NMR (125
RESULT AND DISCUSSION

Synthesis of 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one, 7

The reaction was started by mixing of vanillin, urea and acetone in equivalent molar ratio (Figure 2). This mixture was not completely dissolved, and a few drop of ethanol was added. The reaction was maintained under reflux condition. Monitoring the reaction product was performed using spotting an aliquot sample on TLC plate. Appearing of new spot was an indication the reaction occur. First experiment was accomplished in 8 h. After the reaction was stopped and concentrated in vacuum, direct separation on flash chromatography afforded product in low yield. The un-reacted vanillin was isolated in significant amount as white solid (Table 1). Second experiment was performed by increasing of acetone quantity. It replaced the usage of ethanol as in previous reaction. It was not only homogenized the reaction mixture but also accelerate the reaction time to a half. Beside that, the reaction temperature was also much lower.

![Figure 2. Schematic reaction to yield 7 (left) and ball-stick model structure (right, hydrogen atom was not shown for clarity).](image)

<table>
<thead>
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<th>Table 1. Reaction condition to result product 7</th>
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<tr>
<td><strong>Experiment</strong></td>
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Note: *Heating in water bath

The schematic reaction is displayed as in Figure 2 (left). An equivalent amount of vanillin 6 and urea 3 reacts with acetone 5. The resulted product is dihydropyrimidinone structure 7, and was identified as 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. A class of heterocyclic alkaloid contains diamine (2xNH) in both side of carbonyl (C=O) group and aromatic substituent. Visualisation using 3D molecule model of 7 is showed in Figure 2 (right).
Analysis the molecular structure of 7 was performed using several spectroscopy techniques. Analysis using ultra violet spectrophotometer gave UV absorption spectra with five a maximum wavelength (Figure 3). These spectra characteristic for 3,4-dihydropyrimidinone structure with two N-H amines adjacent to carbonyl groups and an aromatics ring. In addition, infrared spectra recorded significant signal for all the functional groups comprises in the product (Figure 4). Stretching vibration for amine groups appear as double band in 3435.31 and 3358.77 cm\(^{-1}\). Next to it is absorption band for \(=\text{C-H}\) aromatic or double bond and detected at 3218.97 cm\(^{-1}\). The absorption band for stretching vibration of carbonyl amide group was recorded in 1670.30 and 1623.36 cm\(^{-1}\), and also peak 1456.16 cm\(^{-1}\) for methyl group vibration. Furthermore, analysis of the mass spectra using LCMS/MS instrumentation gave a single and clear chromatogram peak at 4.55 min. This peak has molecular weight 235.00 that correspond to molecular mass of the ion from protonated molecule, [M+H]\(^+\). Molecular formula of product 7 is \(\text{C}_{12}\text{H}_{14}\text{N}_{2}\text{O}_{3}\) and has theoretical mass 234.10 atomic unit. This result proved the presence of the isolated product 7 as 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one.

**Proposed reaction mechanism**

The general mechanism of Biginelli reaction was proposed by C. Oliver Kappe in 1997 [19]. It was initiated by interaction of N-urea with an aldehyde formed an iminium ion. This was promoted by acid catalyst protonate the oxygen of the carbonyl group from the aldehyde. This iminium ion further reacts with ethyl-acetoacetate involving its carbon-\(\alpha\) by enolate...
formation. In the mean time, the other side of N-urea attaches to carbonyl group from ethyl-acetoacetate and provides an intermediate structure, called as ureide, which is then constructs a cyclic form and further displace a hydrate producing a dihydropyrimidinone [19]. Recently, Alvim et al. also reported solventless mechanism of the Biginelli reaction [20]. Mechanism was favored following iminium formation as key important step. This adduct is clearly also discovered on MS kinetic study. For this course, iminium intermediate could be afforded by two possible pathways (Figure 5).

First pathway provides the iminium from urea 3 with vanillin 6, and intermediate iminium 8 was afforded. This intermediate theoretically is stabilized by aromatic ring as well as carbamate groups on urea. And the product 7 was easily be formed. On the other side, route 2 was initiated with smaller substrate but less reactive than vanillin. The adjacents α-hydrogen on iminium intermediate 9 stabilize its structure by enamine formation. This route ends up provides dihydropyrimidinone 7. However, stabilization of aromatic ring on iminium 9 was predicted more intent to be favored.

CONCLUSION
An pyrimidinone alkaloid has been synthesized as 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one using naturally abundant vanillin as a yellowish solid in 67% yield.

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REFERENCES


