DIFFERENT ROUTES FOR THE SYNTHESIS OF BENZALDEHYDE-BASED DIHYDROPYIMIDINONES VIA BIGINELLI REACTION

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Abstract

Multicomponent reactions involving three or more reactants are commonly used to prepare dihydropyrimidinone with various bioactivities. This study reports the different routes for the synthesis of benzaldehyde-based dihydropyrimidinone via the Biginelli reaction and investigates the yield of the obtained products. The synthesis was performed via routes A, B, C, D, and E based on the formation of iminium, enamine, and Knoevenagel intermediates between urea, benzaldehyde, and ethyl acetoacetate. Route A, through a one-pot reaction via iminium, produced dihydropyrimidinone with a yield of 58%. The product from route B via iminium was obtained in 62% yield. Route C and D occurred via enamine at room temperature, and reflux gave the product 31% and 40% yield, respectively. Route E involving Knoevenagel intermediate provided the product in a 38% yield. ^{1}H NMR, FTIR, and MS spectroscopic techniques were used for structure elucidation.

***Keywords**: Biginelli reaction, dihydropyrimidinone, iminium*

Introduction

Dihydropyrimidinone (Figure 1a), a six-membered heterocyclic compound having a pyrimidine framework (Figure 1b) with two nitrogen atoms and one carbonyl group, exhibits a broad spectrum of bioactivities ie. anticancer, antitumor, antidiabetic, anti-inflammatory, antibacterial and antimalarial (Chiang *et al*., 2009). The nitrogen atom in the aromatic ring modulates these activities (Manzoor *et al*., 2021; Fadlan *et al*., 2021; Mulyati *et al*., 2022). Dihydropyrimidinone scaffold contained in various commercial drugs and alkaloids such as 5-fluorouracil, idoxuridine, and methylthiouracil, monoastrol, and oxo-monoastrol (Figure 1) (Russowsky *et al*., 2006). Dihydropyrimidinone is effective in health medication and is used to treat and prevent a number of illnesses.

Dihydropyrimidinones are generally prepared from a multicomponent reaction (MCR) involving three or more reactants to produce certain products which contain all the atoms of each reactant. MCR is more efficient and fulfills the atom economy principle compared to linear organic synthesis (Puripat *et al.*, 2015). The application of MCR in the synthesis of dihydropyrimidinone through the Biginelli reaction involves the condensation of aldehydes, urea, and β-keto esters with acid catalysts (Kaur et al., 2017; Nagarajaiah et al., 2016). The Biginelli reaction is simple and easy to produce dihydropyrimidinone and its derivatives. However, the details of the mechanism of this reaction still cannot be determined with certainty even though this reaction has been discovered since 1893.

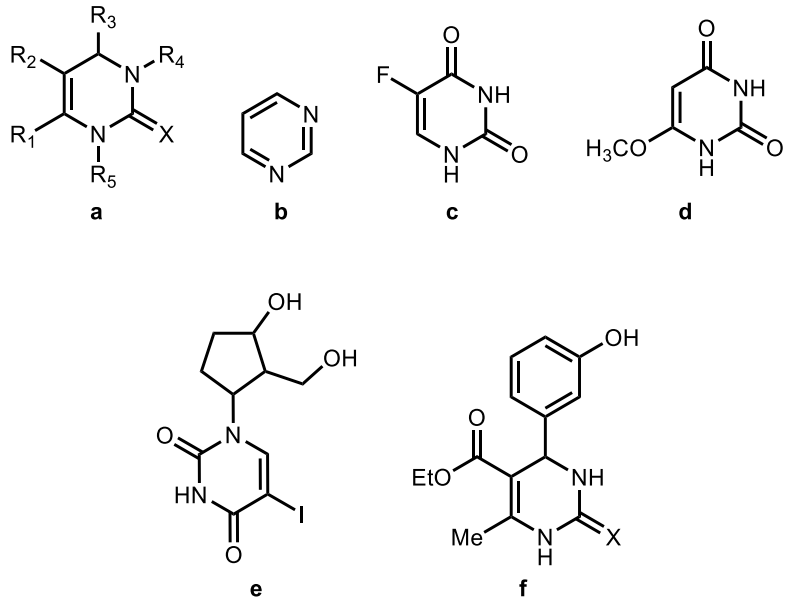

Figure 1. Structure of a six-membered heterocyclic compound. a) pyrimidine, b) dihydropyrimidinone, c) 5-fluorouracil, d) idoxuridine, e) methylthiouracil, f) monoastrol (X=O), oxo-monoastrol (X=S).

Several routes proposed for the mechanism of Biginelli reaction occurring via protonation step have been theoretically reported. Condensation of urea and aldehyde gives carbon nitrogen bond of iminium succeeded by addition of β-keto ester. The enamine route involves the ester and urea reaction producing a C-N enamine bond which then further reacts with the aldehyde. The Knoevenagel reaction as the third proposed route takes place through the reaction of β-keto esters and aldehydes yielding C-C bonds before reacting with urea (Puripat *et al.*, 2015).

The experimental investigation of reaction mechanism has also been reported. Kappe (1997) investigated the Biginelli reaction mechanism through NMR spectroscopy. Monitoring the reaction of aldehyde and β-keto ester in CD_3OH/HCl using ^{1}H and ^{13}C NMR showed that aldol reactions were not occur between the two components at room temperature. The addition of aldehyde and β-keto ester catalyzed by acid was discovered as the rate-determining step (RDS) followed by reaction with urea. De Souza *et al* (2009) identified the intermediates involved in the three proposed route for Biginelli reactions by electrospray ionization mass spectrometry. The study indicated the presence of different intermediates from the three reaction mechanism routes. The results of the study combined with theoretical studies on the carbon carbon and carbon nitrogen bonds concluded that the iminium pathway was preferred over the others. Theoretical study of the Biginelli reaction mechanism has also been investigated by Puripat *et al* (2015). The iminium route was selected as the best route among the three proposed routes as it requires 21.5 kcal/mol lower than the other two routes. However, the influence and relationship of the reaction mechanism route and the yield of the resulting Biginelli products has not been studied so far. This study reports the synthesis of benzaldehyde-based dihydropyrimidinone via the Biginelli reaction using different routes (Figure 2). The research also intends to investigate the relationship between the synthesis route and the yield of the obtained product.

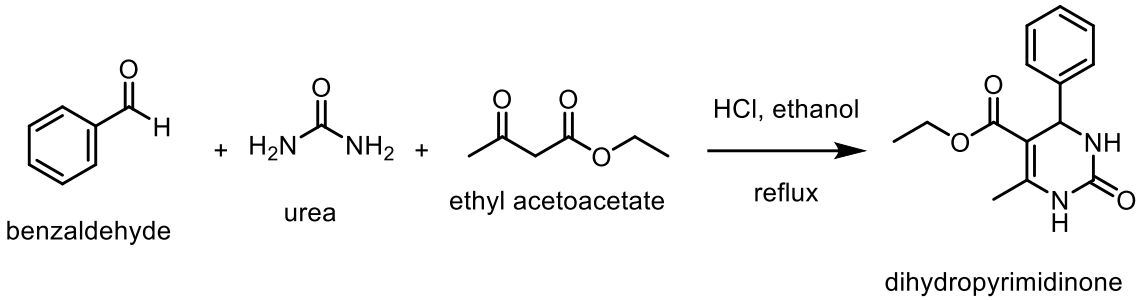

Figure 2. Synthesis of benzaldehyde-based dihydropyrimidinone via the Biginelli reaction

Research Methods

General

Benzaldehyde, urea, and ethyl acetoacetate were in synthetic grade from commercial supplier (Merck, Germany). Other chemicals were in pro analytical grade and distillation was applied for technical grade solvents. The reaction progress was checked by TLC (Merck, Germany). FTIR-8400S spectrometer (Shimadzu, Japan), Bruker Avance Neo-Ascend 500 (Bruker, USA) using DMSO-d_6 and $CDCl_3$ as solvents, and Waters Q-Tof MS Xevo Quadrupole (Waters, USA) were used for structure identification.

Synthesis of dihydropyrimidinone

1) Route A
   Benzaldehyde (0.9975 g, 9.4 mmol), urea (0.5645 g, 9.4 mmol), and ethyl acetoacetate (1.3014 g, 10 mmol) in 10 mL ethanol was treated with 4-5 drops of conc. hydrochloric acid and refluxed until reaction completed (TLC monitor). The mixture was cooled and the precipitated solid was separated by filtration, washed with cold water (3 × 50 mL), dried, and recrystallized (ethanol) to give a white solid (58% yield) (Manzoor et al. 2021).
2) Route B
   Benzaldehyde (0.9975 g, 9.4 mmol) and urea (0.5645 g, 9.4 mmol) in ethanol (4 mL) were treated with 4-5 drops of conc. hydrochloric acid. The reflux was performed for 30 minutes, and ethyl acetoacetate (1.3014 g, 10 mmol) was added. The reflux was done until the reaction completed (TLC monitor) and then allowed to cool (ice bath). The obtained solid was filtered, washed (cold water 3 × 50 mL), dried, and recrystallized (ethanol) to give a white solid (62% yield).
3) *Route C*
   Urea (0.5645 g, 9.4 mmol) and ethyl acetoacetate (1.3014 g, 10 mmol) in ethanol (4 mL) was mixed with 4-5 drops of conc. hydrochloric acid and stirred at r.t. for 30 minutes. The mixture was poured into benzaldehyde (0.9975 g, 9.4 mmol), heated under reflux until the reaction completed (TLC monitor), and then allowed to cool in ice bath. The obtained solid was filtered, washed (cold water 3 × 50 mL), dried, and recrystallized (ethanol) to give a white solid (31% yield).
4) Route D
   Urea (0.5645 g, 9.4 mmol) and ethyl acetoacetate (1.3014 g, 10 mmol) in ethanol (4 mL) was treated with 4-5 drops of conc. hydrochloric acid. The mixture was refluxed for 30 minutes, and added with benzaldehyde (0.9975 g, 9.4 mmol). The mixture was further refluxed (TLC monitor) and then allowed to cool (ice bath). The precipitated solid was separated by filtration, washed (cold water 3 × 50 mL), dried, and recrystallized (ethanol) to give a white solid (40% yield).
5) *Route E*
   Benzaldehyde (0.9975 g, 9.4 mmol) and ethyl acetoacetate (1.3014 g, 10 mmol) in ethanol (4 mL) was treated with conc. hydrochloric acid (4-5 drops) and refluxed (30 minutes). The mixture was further refluxed (TLC monitor) after the addition of urea

(0.5645 g, 9.4 mmol) and then allowed to cool in ice bath. The obtained solid was filtered and washed (cold water 3 × 50 mL), dried, and recrystallized (ethanol) to give a white solid (38% yield).

Results and Discussion

In this study, the ethyl ester of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimi-dine-5-carboxylate was obtained through five different routes. The product was obtained in 58%, 62%, 31%, 40%, 38% yield, respectively, for routes A, B, C, D, and E (Table 1). The different routes affect the yield of the resulting product compound.

Table 1. % Yield of compound from different routes

Routes	% Yield
A	58%
B	62%
C	31%
D	40%
E	38%

The synthesis of benzaldehyde-based dihydropyrimidinone through five routes was performed based on the different intermediate formed during the reaction occurs. Reactions via routes A and B involve the formation of iminium, whereas routes C and D occur via enamines. The reaction through the E route takes place by intermediary Knoevenagel compounds (Figure 3) (Puripat *et al.*, 2015). The synthesis of compound was carried out in ethanol with the addition of concentrated hydrochloric acid as catalyst. Benzaldehyde, urea, and ethyl acetoacetate were altogether mixed under reflux conditions in route A. Benzaldehyde and urea were firstly mixed and then followed by the addition of ethyl acetoacetate in route B. Route C was executed by mixing urea and ethyl acetoacetate at r.t. superseded by addition of the mixture to benzaldehyde, while urea and ethyl acetoacetate were mixed under reflux and benzaldehyde was added to the mixture in route D. Furthermore, route E involved mixing benzaldehyde and ethyl acetoacetate under reflux and the addition of urea to the mixture. The formation of dihydropyrimidinone was continued through reflux and monitored by TLC.

The TLC monitor as shown in Figure 4 indicates the formation of intermediates with different Rf values than that for benzaldehyde and ethyl acetoacetate as the reaction running for 30 minutes. Routes A, B, and D gave relatively clean reaction (Figure 4a, 4b, 4d), while routes C and E with benzaldehyde and ethyl acetoacetate (Figure 4c and 4e). This monitoring correlates with the % yield of compound produced from each route.

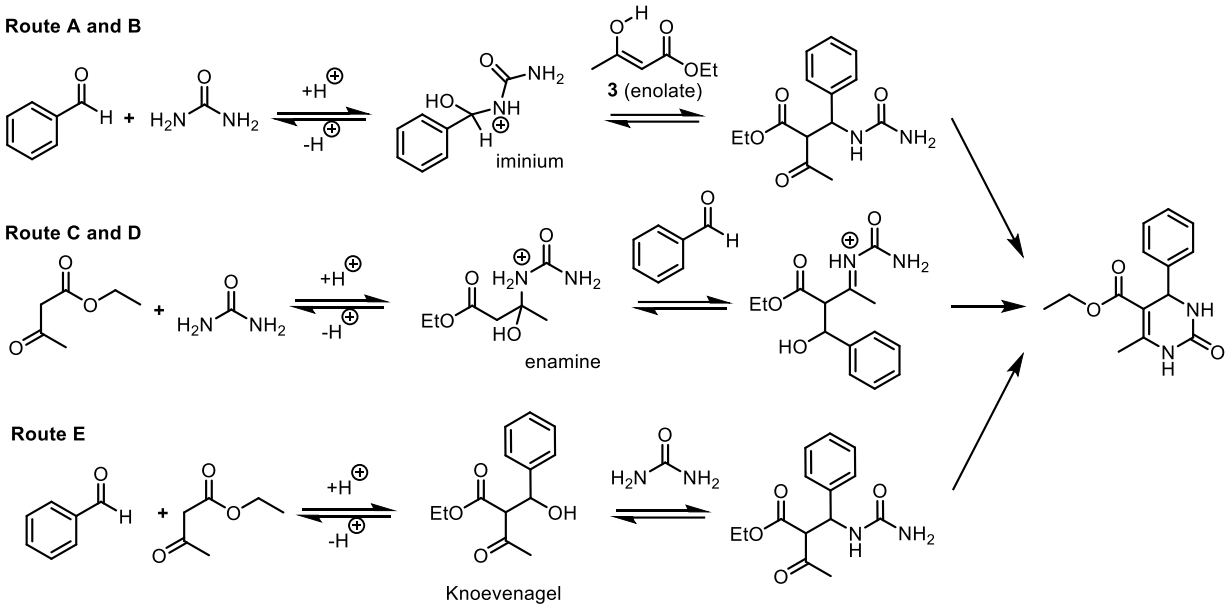

Figure 3. The synthesis routes for benzaldehyde-based dihydropyrimidinone

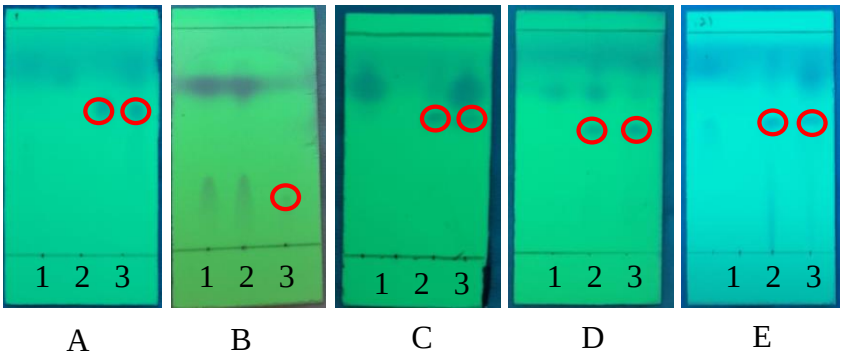

Figure 4. TLC monitors the formation of intermediates for 30 minutes reaction (*n*-hexane:ethyl acetate 1:1). Routes a) A, b) B, c) C, d) D, e) E. 1 = benzaldehyde; 2 = ethyl acetoacetate; 3 = mix of 1, 2, 4; 4 = reaction mixture.

The TLC monitor indicates the reaction of route A gave the product in 30 minutes of reaction as denoted by a new spot with different Rf value compared to benzaldehyde and ethyl acetoacetate. A white solid was established after 60 minutes and then the reaction was stopped in 120 minutes of reaction indicated by the disappearance of the ethyl acetoacetate in TLC. Route B was executed by mixing benzaldehyde and urea under reflux conditions and a white precipitate began to form within 30 minutes of reaction time after the addition of ethyl acetoacetate. The reaction was stopped when the ethyl acetoacetate disappeared after 60 minutes of reaction. The rate determining step in route B occurs during the C-N bond formation with lower energy (21.5 kcal mol^{-1}) contrasted to the other routes (Puripat *et al.*, 2015). This correlates with the % yield of product which is 62%.

Route C via enamine was started by mixing urea and ethyl acetoacetate at room temperature, while in route D the mixing process of ethyl acetoacetate and urea was done under reflux. Based on the % yield of product which is 31% for route C and 40% for route D, the formation of enamine is affected by temperature. In routes C and D, ethyl acetoacetate undergoes a keto-enol tautomerization

and the formation of enol and its stabilization from interaction with solvent and the acid catalyst are important for Biginelli reaction. The enolate is more active so that it determines the rate-determining step of the reaction which affects the formation of the final product (Freitas *et al.*, 2019). The route D reaction carried out at a higher temperature causes the tautomerization of ethyl acetoacetate faster and more abundant than reaction via route C (Puripat *et al.*, 2015). The reaction product started to form in 60 minutes of reaction according to TLC monitor via routes C and D. The precipitate was formed after 60 and 40 minutes of reaction, respectively. The reaction was stopped at 120 minutes of reaction after the disappearance of ethyl acetoacetate.

In route E, the Knoevenagel intermediate was formed by mixing benzaldehyde and ethyl acetoacetate under reflux conditions. The Knoevenagel compound is reported in *cis*- and *trans*-isomers and they rise with increasing the reaction temperature (Sahota *et al.*, 2019). The reaction of route E gave the reaction product after 15 minutes of reaction marked by the appearance of a new spot with different Rf value from reactans. The formation of Knoevenagel intermediates in route E is the RDS for the formation of product. Furthermore, a white solid was obtained after 40 minutes. The reaction was stopped when the ethyl acetoacetate spot disappeared after 60 minutes of reaction.

Identification of compound structure

6-Methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester. Method A: white solid (58%), method B: white solid (62%), method C: white solid (31%), method D: white solid (40%), method E: white solid (38%). Rf = 0,56 (*n*-hexane:ethyl acetate 1:1); melting point 239-240ºC; IR (KBr disc) ν_{max} 3244, 3188, 1724, 1703, 1645, 1290, 1092 cm^{-1}; ^1H NMR (500 MHz, DMSO-*d6* + $CDCl_3$) δ 9,23 (s, 1H), 7,77 (s, 1H), 7,34–7,23 (m, 5H), 5,14 (d, J = 3 Hz, 1H), 4,00 (q, J = 8 Hz, 2H), 2,25 (s, 3H), 1,10 (t, J = 7 Hz, 3H); HRMS (ESI-TOF) $C_{14}H_{17}N_2O_3$ $[M+H]^+$ calc. 261,1239, found 261,1223.

Structure identification of ethyl ester 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate with NMR spectrometer (DMSO-d_6 + $CDCl_3$ solvent, 500 MHz) gave a ^1H NMR spectrum with seven types of protons; a triplet signal at chemical shifts (δ) of 1.10 ppm attributed for the methyl proton of ethyl acetoacetate unit, a singlet at δ 2.25 ppm corresponds to the proton of methyl pyrimidine unit, a quartet at δ 4.00 ppm matches for the protons of methylene group coupled by three methyl group protons, a doublet at δ 5.14 for methine proton, a multiplet at δ 7.23-7.34 ppm correlates for aromatic protons, and two singlet signals at δ 9.23 ppm and δ 7.77 ppm for two protons of the NH groups.

The identification of benzaldehyde-based dihydropyrimidinone by NMR was supported by infrared and mass spectra. The infrared spectrum of compound shows absorption at wave numbers (ν) of 3244 cm^{-1} and 3177 cm^{-1} suitable for NH group. The band at ν 1703 cm^{-1} and 1724 cm^{-1} are typical for the C=O group, while the band at ν 1645 cm^{-1} attributes for the C=C group. A typical absorption at ν 1290 cm^{-1} and 1092 cm^{-1} corresponds for the C-O bond. The title compound gives the $[M]^+$ ion band at m/z 261.1223 with a molecular formula of $C_{14}H_{17}N_2O_3$ correlates to the theoretical m/z of 261.1239.

Conclusions

The ethyl ester of benzaldehyde-based dihydropyrimidinone has been obtained by the Biginelli reaction through different routes. Route A and B by iminium gave compound with 58% and 62% yields. Route C and D through the enamine and route E occured by Knoevenagel produce compound with 31%, 40% and 38% yields. Spectroscopic techniques NMR,

FTIR, and MS confirmed the dihydropyrimidinone.

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