Chimica et Natura Acta
p-ISSN: 2355-0864 e-ISSN: 2541-2574
Homepage: http://jurnal.unpad.ac.id/jcena

Study and Characterization of Nitration of Isovanillic Acid Derivatives using NMR and Mass Spectroscopy

Elvira Hermawati[1]*, Pipih Lutfi Sa'adah[1], Ade Danova[1], Didin Mujahidin[1], Iqbal Musthapa[2]

[1]Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Jl. Ganesha No. 10, Coblong Bandung, 40132, Indonesia.
[2]Department of Chemistry, Faculty of Education of Mathematical and Natural Sciences, Universitas Pendidikan Indonesia, Jl. Dr. Setiabudi No.229, Bandung 40154, Indonesia.
*Corresponding author: elvira.hermawati@itb.ac.id

DOI: https://doi.org/10.24198/cna.v13.n3.62271

Abstract: Isovanillic acid and its derivatives serve as precursors in the synthesis of EGFR tyrosine kinase inhibitors, which are used to treat cancer cell lines. A crucial step in this process is the nitration of isovanillic acid through nucleophilic aromatic substitution, resulting in 6-nitroisovanilic acid and its derivatives, which act as intermediates for forming a quinazolinone ring. However, this study revealed that direct nitration of isovanillic acid derivatives led to unexpected products, such as 3-hydroxy-4-methoxy-2,6-dinitrobenzoic acid **(1)** and 4-(3-(2-methoxy-4-nitrophenoxy)propyl)morpholine **(4)**. Additionally, the optimal conditions for etherification of 2 with N-(3-chloropropyl)morpholine to produce **3** involved using Cs2CO3 in DMF and refluxing for 7 hours, achieving an 89% yield. All synthesized compounds were characterized using NMR spectroscopy, and mass spectrometry was employed for two compounds **(3, 4)**. Compound **1** represents the first report of direct nitration of isovanillic acid. Compound **4** was synthesized for the first time from 3 through a one-pot process involving hydrolysis and decarboxylation, followed by nitration at carbon C-1 without metal catalysis, as confirmed by a NOESY 1D experiment. Moreover, the application of **4** could hold promise for future advancements in medicinal chemistry.

Keywords: EGFR inhibitor, etherification, gefitinib, isovanillic acid, nitration

Abstrak: *Asam isovanilat dan turunannya berfungsi sebagai prekursor dalam sintesis penghambat tirosin kinase EGFR, yang digunakan untuk menghambat berbagai sel kanker. Tahapan penting dalam proses ini adalah nitrasi asam isovanilat melalui substitusi aromatik nukleofilik, menghasilkan asam 6-nitroisovanilat dan turunannya, yang berperan sebagai intermediet dalam pembentukan cincin kuinazolinon. Namun, penelitian ini menunjukkan bahwa nitrasi langsung terhadap turunan asam isovanilat menghasilkan produk yang tidak terduga, yaitu asam 3-hidroksi-4-metoksi-2,6-dinitrobenzoat (1) dan 4-(3-(2-metoksi-4-nitrofenoksi)propil)morfolina (4). Selain itu, kondisi optimal untuk eterifikasi senyawa 2 dengan N-(3-kloropropil)morfolina untuk menghasilkan senyawa 3 melibatkan penggunaan Cs_2CO_3 dalam DMF dan refluks selama 7 jam, dengan hasil rendemen sebesar 89%. Semua senyawa hasil sintesis dilakukan karakterisasi menggunakan spektroskopi NMR, dan spektrometri massa untuk dua senyawa (3, 4). Senyawa 1 untuk pertama kalinya dilaporkan dengan nitrasi langsung terhadap asam isovanilat. Senyawa 4 disintesis untuk pertama kalinya dari senyawa 3 melalui proses satu wadah (one-pot) yang melibatkan reaksi hidrolisis dan dekarboksilasi, diikuti nitrasi pada karbon C-1 tanpa katalis logam, sebagaimana dikonfirmasi oleh eksperimen NOESY 1D. Lebih lanjut, penerapan senyawa 4 berpotensi menjanjikan untuk pengembangan lebih lanjut di bidang kimia medisinal.*

Kata kunci: *penghambat EGFR, eterifikasi, gefitinib, asam isovanilat, nitrasi*

INTRODUCTION

Isovanillic acid is primarily produced through the depolymerization of lignin and the oxidation of isovanilline (Irmak *et al*. 2020, Shan *et al*. 2016). Numerous studies have demonstrated that isovanillic acid exhibits a broad range of biological activities (Silva *et al*. 2021; Choi & Kim 2022; Chen *et al*. 2023). Additionally, isovanillic acid serves as an essential precursor in the creation of quinazolinone derivatives. These derivatives have been the focus of extensive research as potential anticancer agents due to their strong ability to inhibit various receptor tyrosine kinases, such as EGFR or VEGFR-2, which are often overexpressed or dysregulated in numerous solid tumors (Kumar *et al*. 2014, Agarwal *et al.* 2007, Hennequin *et al*. 2002, Herbst *et al*. 2004).

Among the quinazolinone derivatives, Erlotinib (Tarceva), Gefitinib (Iressa), and Vandetanib (Zactima) were recently licensed by the FDA as anticancer medications (Figure 1) (Dowell *et al*. 2005, Herbst *et al*. 2004, Herbst *et al*. 2007, Gibson 1998, Cohen *et al*. 2003. They work as ATP-mimetic inhibitors, and many research papers and patents in recent years have addressed their production, action, and therapeutic applications (Marzaro *et al*. 2010; Shaik *et al*. 2023; Rewcastle & Showalter 2000).

Despite their extensive applicability, the reported syntheses of quinazolinone derivatives need multistep and low-yielding techniques (Marzaro *et al*. 2010; Knesl *et al*. 2006). Quinazolinone derivatives have been created through multistep reactions involving various precursors, including isovanillin, isovanillic acid, and 3,4-dimethoxybenzaldehyde (Chandregowda *et al*. 2007; Li *et al*. 2007; Marzaro *et al*. 2010). In earlier research, the crucial step with isovanillic acid involved the nitration of its derivatives, such as methyl 4-methoxy-3-benzyloxybenzoate or methyl 4-methoxy-3-(3-chloroporpyloxy)benzoate, which served as precursors for quinazolinone production (Kumar *et al*. 2014). Therefore, our research is focused on evaluating the nitration conditions for isovanillic acid and its derivatives. Moreover, all products were characterized using NMR spectroscopy (NOESY 1D) and mass spectrometry to confirm their structures.

MATERIALS AND METHOD

The Instruments and Materials

Chemicals were sourced from Sigma Aldrich and TCI, including 3-hydroxy-4-methoxybenzoate, N-(3-chloropropyl)morpholine, 1-bromo-3-chloropropane, benzyl bromide, HNO_3 65%, $SOCl_2$, AcOH, Ac_2O, K_2CO_3, Cs_2CO_3, and aqua DM. During the synthesis phase, pro-analytical (p.a) solvents such as methanol, acetonitrile, acetone, and DMF were utilized. The Agilent DD2 system was employed to characterize all synthesized compounds, operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. High-resolution electrospray ionization mass spectrometry (ESI-TOF) data was acquired using the Waters LCT Premier XE mass spectrometer.

Synthesis 3-hydroxy-4-methoxy-2,6-dinitrobenzoic acid (1)

Isovanillic acid (500 mg, 2.974 mmol, 1 equivalent) was introduced, and 65% HNO_3 was gradually added while maintaining the temperature between 0 and 5°C in an ice bath. Subsequently, cold aqua DM was incorporated into the reaction mixture, which was then separated by filtration through a Büchner funnel, resulting in a yellow product without further purification.

^{1}H-NMR $(CD_3)_2CO$): δ_H (ppm) 7.86 (s, 1H), 4.13 (s, 3H). ^{13}C-NMR $(CD_3)_2CO$): δ_c (ppm) 163.3, 150.5, 145.3, 139.6, 118.2, 109.7, 57.8.

Synthesis methyl 3-hydroxy-4-methoxybenzoate (2)

To prepare the reaction, 5 grams (0.029 mol, 1 equivalent) of isovanillic acid were dissolved in 250 ml of methanol. Thionyl chloride ($SOCl_2$, 6.5 mL, 0.089 mol, 3 equivalents) was then gradually added to the solution while it was kept in an ice bath. The resulting mixture was stirred and subjected to reflux for a duration of 3 hours. Following this, the reaction was halted and allowed to cool to room temperature. The mixture was then treated with 100 mL of aqua DM and NaOH until the pH reached approximately 2. It was subsequently evaporated under vacuum, and the water and organic phases were separated. The organic phase was cooled, yielding a purple-black product with a yield of 79.6%.

^{1}H-NMR ($CDCl_3$): δ_H (ppm) 3.88 (s, 3H), 3.94 (s, 3H), 6.87 (d, 1H, J = 8.3 Hz), 7.58 (d, 1H, J = 1.9 Hz), 7.60 (dd, 1H, J = 1.9 & 8.3 Hz). ^{13}C-NMR ($CDCl_3$): δ_c (ppm) 166.8, 123.9, 115.6, 150.8, 145.2, 109.8, 122.8, 56.0, 51.9.

Synthesis methyl 4-methoxy-3-(3-morpholinopropoxy) benzoate (3)

Methyl 3-hydroxy-4-methoxybenzoate (2) (40 mg, 0.219 mmol, 1 equivalent) was dissolved in either acetone or DMF. Subsequently, 2 equivalents of the base and *N*-(3-chloropropyl)morpholine (72 mg, 0.439 mmol, 2 equivalents) were introduced and the mixture was refluxed. Following the reaction, cold aqua DM was added to the mixture, which was then filtered through a Büchner funnel to yield a brownish residue. This residue was extracted with n-hexane and filtered again using a Büchner funnel. The resulting product was a light brown solid.

^{1}H-NMR ($CDCl_3$): δ_H (ppm) 7.56 (s, 1H), 6.88 (d, 1H, J = 8.4 Hz), 7.67 (d, 1H, J = 8.4 Hz), 3.91 (s, 3H), 3.89 (s, 3H), 4.14 (t, 2H, J = 6.6 Hz), 2.04 (q, 2H), 2.53 (t, 2H, J = 7.1), 2.47 (s, 4H), 3.72 (t, 4H, J = 4.4 Hz). ^{13}C-NMR ($CDCl_3$): δ_c (ppm) 166.9, 123.6, 110.5, 148.0, 153.3, 113.6, 122.6, 67.3, 67.0, 56.0, 55.4, 53.7, 51.9, 26.2. TOF MS ESI: *m/z* $[M+H]^+$: 310.1648 (calculated *m/z* $[M+H]^+$ for ion $C_{16}H_{24}NO_5$: 310.1654 *m/z*).

Synthesis of 4-(3-(2-methoxy-4-nitrophenoxy)propyl)morpholine (4)

Methyl 4-methoxy-3-(3-morpholinopropoxy) benzoate (**3**) (100 mg, 0.323 mmol, 1 equivalent) was added and then HNO_3 65% (572 µL, 11.365 mmol, 36 equivalents) dropwise in the ice bath at temperature 0 – 5 °C for 5 hours. Then the reaction mixture was added with cold aqua DM and NaOH untill pH ~ 7 and filtered using a Büchner funnel. A yellow product was obtained.

^{1}H-NMR (D_2O): δ_H (ppm) 7.77 (1H, d, J = 8.4 Hz), 7.69 (1H, s), 7.28 (1H, d, J = 8.4 Hz),

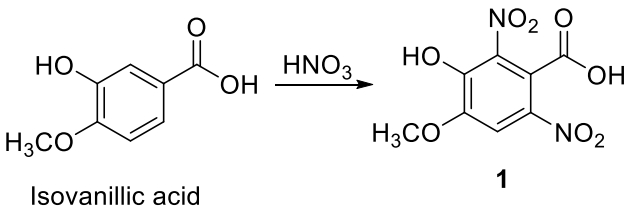
4.39 (2H, t, J = 6.0), 4.11 (7H, s), 3.27 (4H, t, J = 8.7), 2.36 (2H, t, J = 7.3), 2.15 (2H, s).

^{13}C-NMR (D_2O): δ_c (ppm) 151.2, 146.5, 129.23, 123.5, 114.1, 111.1, 67.1, 65.0, 56.2, 55.3, 52.3, 24.0. MS-TOF-ESI: *m/z* $[M+H]^+$: 297.1444 (calculated *m/z* $[M+H]^+$ for ion $C_{14}H_{20}N_2O_5$: *m/z* 297.1450).

RESULT AND DISSCUSION

The first step of quinazolinone synthesis is the nitration process on isovanillic acid through nucleophilic aromatic substitution to produce 6-nitroisovanilic acid. In our initial experiment, isovanillic acid underwent nitration using either 65% HNO_3 or a mixture, with the temperature maintained between 0-5 °C. According to Table 1, when isovanillic acid was treated with 24 equivalents of 65% HNO_3, the yield was 25.9%. NMR spectroscopy was employed to elucidate the structure of the resulting product. The ^{1}H-NMR spectrum revealed two singlet signals at 7.86 and 4.13 ppm, corresponding to the aromatic proton and methoxy group, respectively. This suggested that the aromatic ring was substituted with five functional groups. Consequently, the nitration process yielded 3-hydroxy-4-methoxy-2,6-dinitrobenzoic acid **(1)**. Moreover, the nitric acid to isovanillic acid ratio was reduced to 12 equivalents, or acid mixtures (HNO_3:AcOH:Ac_2O) were used in a 5:15:3 ratio. Nevertheless, the product remained 3-hydroxy-4-methoxy-2,6-dinitrobenzoic acid, with yields of 49.8% and 21.5% for 12 equivalents of 65% HNO_3 and the acid mixtures, respectively. However, employing 100% HNO_3 or a combination of KNO_3 and H_2SO_4 resulted in no product formation, as indicated in Table 1. As presented in Scheme 2, this result demonstrated that 2,6-dinitro compound (**1**) could not be avoided despite the declining ratio of nitric acid and by mixing with another acid, such as acetic acid and acetic anhydride because isovanillic acid has free hydroxy group attached to the aromatic ring at 3 position which is very active as an *ortho* and *para* direction in electrophilic substitution of the aromatic ring. As a previous report, compound **1** was synthesized through two steps of (Greenwood & Robinson 1932) nitration. Therefore, compound **1** was the first report from direct nitration of isovanillic followed by determining the structure using NMR spectroscopy.

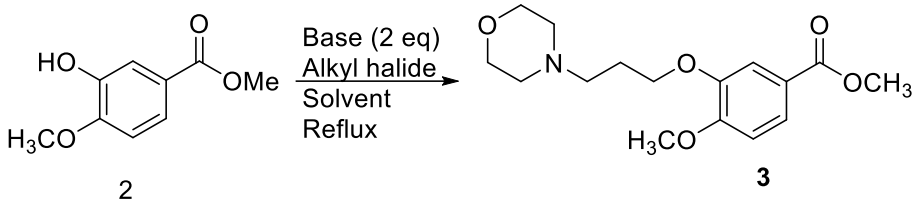
In order to advance our research, we conducted an esterification reaction on isovanillic acid using thionyl chloride in methanol. The resulting pure ester product **(2)** was then subjected to a reaction with *N*-(3-chloropropyl)morpholine through an ether Williamson reaction under basic and reflux conditions, as depicted in Scheme 2. The purpose of etherifying the hydroxy group was to limit ortho directionality by introducing an alkyl group such as methyl, benzyl, or acyl. Additionally, employing *N*-(3-chloropropyl)morpholine in this study aimed to streamline the synthesis of quinazolinone derivatives like gefitinib. As shown in Table 2, the optimal condition for etherification with *N*-(3-chloropropyl)morpholine involved using cesium carbonate (Cs_2CO_3) in DMF, refluxed for 7 hours, yielding a product with an 89% yield. This was better to other conditions, such as K_2CO_3 with acetone, Cs_2CO_3 with acetone, and K_2CO_3 with DMF, which required 18-24 hours and resulted in yields of 37.9-48.5% (Table 2). This suggests that Cs_2CO_3 in DMF is a favorable environment for conducting the bimolecular nucleophilic substitution (S_N2) due to the more basic and stable phenol ion in an aprotic solvent. The product was verified using NMR spectroscopy. The ^{1}H-NMR spectrum revealed methylene peaks at 4.14 ppm attached to oxygen, 2.53 ppm attached to nitrogen, and 2.04 ppm between two methylene groups of propyl. The ^{13}C-NMR spectrum indicated the presence of the ether group from propyl at 67.3 ppm. These findings.



Scheme 1. Synthesis of 3-hydroxy-4-methoxy-2,6-dinitrobenzoic acid (**1**)

Table 1. Screening of nitration condition on isovanillic acid

No.	Acid	Equivalent	Temp (°C)	Time (h)	Yield (%)
1	HNO_3 65%	24	0-5	3	25.9
2	HNO_3 65%	12	0-5	3	49.8
3	HNO_3 65%:AcOH:Ac_2O	5:15:3	r.t	5	21.5
4	HNO_3 100%	24	0-5	5	no reaction
5	KNO_3 : H_2SO_4	2:31	60	5	no reaction

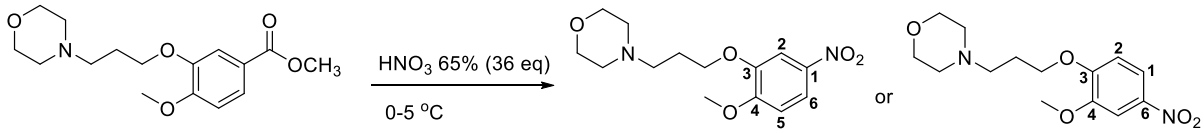


2 **3**

Scheme 3. Synthesis of methyl 4-methoxy-3-(3-molfolinopropoxy) benzoate (**3**)

Table 2. Optimization of compound **3**

No	Base	Solvent	Time (h)	Product (mg)	Yield (%)
1	K_2CO_3	Acetone	24	29.2	43
2	Cs_2CO_3	Acetone	48	25.7	37.9
3	K_2CO_3	DMF	18	33.0	48.5
4	Cs_2CO_3	DMF	7	60.5	89



3 **4**

Scheme 4. Synthesis of 4-(3-(2-methoxy-4-nitrophenoxy)propyl)morpholine **(4)**

Table 3. Screening of nitration condition on compound **3**

No.	Acid	Equivalent	Temp (°C)	Time (h)	Product
1	HNO_3 65%	12	0-5	12	no reaction
2	HNO_3 65%	24	0-5	8	no reaction
3	HNO_3 65%	36	0-5	5	81%
4	HNO_3 65%: H_2SO_4 98%	2:9	r.t	24	no reaction
5	HNO_3 65%: AcOH	9:14	r.t	24	no reaction
6	HNO_3 65%:AcOH:Ac_2O	15:15:3	r.t	24	no reaction
7	HNO_3 65%:AcOH:Ac_2O	20:30:3	60	5	no reaction

confirm the successful formation of the etherification product (Gilday *et al*. 2016, Shih *et al*. 2011).

Additionally, the nitration of compound **3** was carried out using 65% HNO_3, as previously outlined in Table 1 and depicted in Scheme 4. Table 3 indicates that various nitration conditions were tested on compound **3**, but only the application of 36 equivalents of 65% HNO_3 resulted in product formation. This process involved the gradual addition of 36 equivalents of 65% HNO_3 at a temperature range of 0-5°C over a period of 5 hours. Upon completion of the reaction, as monitored by thin layer chromatography, the reaction mixture was processed, and the resulting product was neutralized by adding cold water and sodium hydroxide until a pH of 7 was achieved. The precipitated product was then filtered and dried, yielding an 81% recovery. Subsequently, NMR spectroscopy was conducted on the purified product. The ^{1}H-NMR spectrum revealed an ABX system at 7.77 (1H, d, *J*= 8.4 Hz), 7.69 (1H, s), and 7.28 (1H, d, *J*= 8.4 Hz) ppm, suggesting two possibilities: either without nitration or with nitration followed by a decarboxylation process. Therefore, further analysis was performed using 13C-NMR spectroscopy and mass spectrometry. The ^{13}C-NMR spectrum showed no carbonyl signal around 166.9 ppm, which was corroborated by mass spectrometry with *m/z* $[M+H]^+$ = 297.1444 (calculated for $C_{14}H_{20}N_2O_5$ = 297.1450). This confirmed that the nitration of compound 3 resulted in the formation of an ipso product (Rakshit *et al*. 2018), specifically 4-(3-(2-methoxy-4-nitrophenoxy)propyl)morpholine **(4)**. Furthermore, the NOESY 1D experiment was performed to know the regioselectivity of nitration whether nitro group attached to carbon at 1 position or at 6 positions, as presented in scheme 4. Thus, the

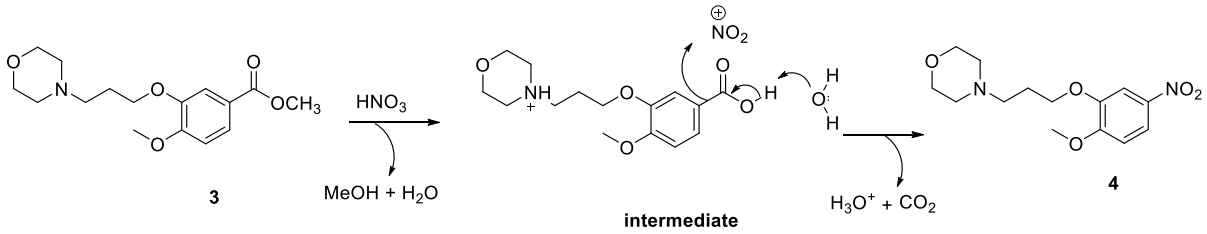


Figure 1. Proposed mechanism of nitration through metal-free decarboxylation in a one-pot process

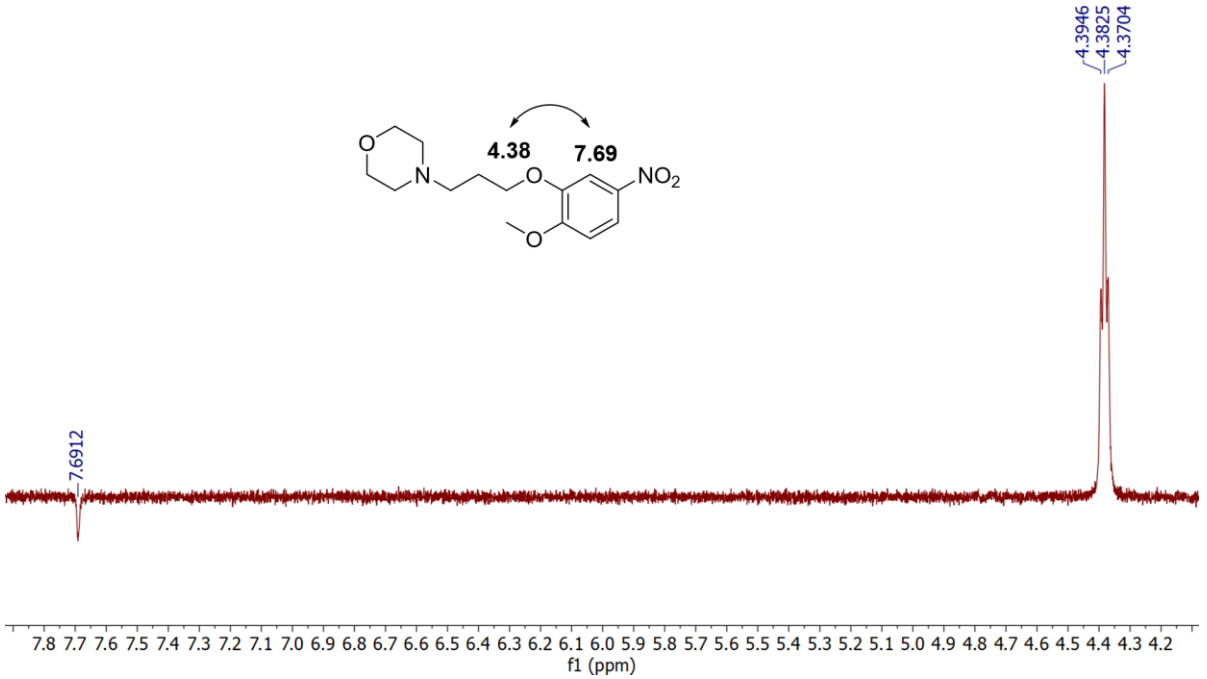


Figure 2. NOESY 1D experiment of compound **4**

proton signal at 7.69 (s, 1H) was chosen and treated. As presented in Figure 2, the result revealed that the proton signal at 7.69 (1H, s) showed a correlation with proton at 4.38 (t, 2H) belonging to -CH_2-O of propyl morpholine. This proved that the nitro group was at 1 position, as presented in Figure 1.

Unexpectedly, compound **4** was initially created through a process of hydrolysis and decarboxylation, followed by direct nitration without the use of metal catalysts, as depicted in Figure 1. In previous research (Hoang *et al*. 2017), compound **4** served as an intermediate in the synthesis of a human glutaminyl cyclase inhibitor. Additionally, the carboxylation process took place on 2,4-dimethoxybenzoic acid and 3-(benzyloxy)-4-methoxybenzaldehyde due to the severe acidic conditions (Howe *et al*. 2016, Rakshit *et al*. 2018). According to a literature review (Nishino *et al*. 2013; Kumar *et al*. 2014), there is no documented instance of a decarboxylation product resulting from the nitration of methyl 4-methoxy-3-benzyloxybenzoate or methyl 4-methoxy-3-(3-chloroporpyloxy)benzoate under harsh acidic conditions to produce 6-nitroisovanilic acid, as illustrated in Scheme 1. This suggests that the decarboxylation might be attributed to the morpholine moiety present in compound **3**, which could become protonated in harsh acidic conditions, forming ammonium ions during the nitration process, as shown in Figure 1. These ammonium ions would be more soluble in polar conditions, potentially facilitating the hydrolysis and decarboxylation process under harsh acidic conditions through intermediate to yield compound **4**, as depicted in Figure 1. Therefore, it remains necessary to screen the nitration conditions for compound 3 to prevent decarboxylation by employing milder conditions during the nitration process.

CONCLUSION

In conclusion, attempts to directly nitrate isovanilic acid derivatives (**1**, **3**) did not yield 6-nitroisovanillic acid. Additionally, four compounds (**1**-**4**) were synthesized from isovanillic acid in this research. The optimal nitration condition for isovanillic acid involved using 12 equivalents of 65% HNO_3 for 3 hours, resulting in a 49.8% yield for compound **1**. The best conditions for etherification between *N*-(3-chloropropyl)morpholine and methyl 3-hydroxy-4-methoxybenzoid acid were achieved with Cs_2CO_3 in DMF, refluxed for 7 hours, yielding 89% for compound **3**. Compounds **1** and **4** were

synthesized for the first time derived from isovanillic acid. Compound **4** shows potential for further exploration in medicinal chemistry through the formation of its derivatives, and there remains a need for the development of new mild nitration and environmentally friendly processes to optimize the synthesis of gefitinib.

ACKNOWLEDGMENTS

The author extends his heartfelt appreciation to Prof. Yana Maolana Syah, MS, and the integrated chemistry laboratory at the Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung.

REFERENCES

Agarwal, P.K., Black, P.C., McConkey, D.J. & Dinney, C.P. (2007). Emerging drugs for targeted therapy of bladder cancer. *Expert Opinion on Emerging Drugs*. **12(3)**: 435-448.

Chandregowda, V., Venkateswara Rao, G. & Chandrasekara Reddy, G. (2007). Convergent approach for commercial synthesis of gefitinib and erlotinib. *Organic Process Research & Development*. **11(5)**: 813-816.

Chen, X., Hu, C., Shu, Z., Wang, X., Zhao, Y., Song, W., Chen, X., Jin, M., Xiu, Y., Guo, X, Kong, X., Jiang, Y,m Guan, J., Wang, L. & Wang, B. (2023). Isovanillic acid protects mice against Staphylococcus aureus by targeting vWbp and Coa. *Future Microbiology*. **18(11)**: 735-749.

Choi, J.H. & Kim, S. (2022). In vitro antithrombotic, hematological toxicity, and inhibitor studies of protocatechuic, isovanillic, and p-hydroxybenzoic acids from *Maclura tricuspidata* (Carr.) Bur. *Molecules*, **27(11)**: 3496.

Cohen, M.H., Williams, G.A., Sridhara, R., Chen, G. & Pazdur, R. (2003). FDA drug approval summary: gefitinib (ZD1839)(Iressa®) tablets. *The Oncologist*. **8(4)**: 303-306.

Dowell, J., Minna, J.D. & Kirkpatrick, P. (2005). Erlotinib hydrochloride. *Nature Reviews Drug Discovery*. **4(1):** 13-14.

Gibson, K.H. (1998). *Quinazoline derivatives*. US Patent No. 5770599.

Gilday, J.P. & David, M. (2006). *Process for the preparation of 4-(3'-chloro-4'-fluoroanilino)-7-methoxy-6- (3-morpholinopropoxy)quinazoline*. WO2004024703.

Greenwood, M. & Robinson, R. (1932). 179. β-Pseudognoscopine. J*ournal of the Chemical Society (Resumed)*. **0**: 1370-1376.

Hennequin, L.F., Stokes, E.S.E., Thomas, A.P., Johnstone, C., Ple, P.A., Ogilvie, D.J., Dukes, M., Wedge, S.R., Kendrew, W,J. & Curwen, J.O. (2002). Novel 4-anilinoquinazolines with C-7 basic side chains: design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *Journal of Medicinal Chemistry*. **45(6)**: 1300-1312.

Herbst, R.S., Fukuoka, M. & Baselga, J. (2004). Gefitinib—a novel targeted approach to treating cancer. *Nature Reviews Cancer*. **4(12)**: 956-965.

Herbst, R.S., Heymach, J.V., O'Reilly, M.S., Onn, A. & Ryan, A.J. (2007). Vandetanib (ZD6474): an orally available receptor tyrosine kinase inhibitor that selectively targets pathways critical for tumor growth and angiogenesis. Expert Opinion on Investigational Drugs. **16(2)**: 239-249.

Hoang, V.H., Tran, P.T., Cui, M., Ngo, V.T.H., Ann, J., Park, J., Lee, J., Choi, K., Cho, H., Kim, H., Ha, H.J., Hong, H.S., Choi, S., Kim, Y.H. & Lee, J. (2017). Discovery of potent human glutaminyl cyclase inhibitors as anti-Alzheimer's agents based on rational design. Journal of Medicinal Chemistry. **60(6)**: 2573-2590.

Howe, G.W., Vandersteen, A.A. & Kluger, R. (2016). How acid-catalyzed decarboxylation of 2, 4-dimethoxybenzoic acid avoids formation of protonated CO_2. *Journal of the American Chemical Society*. **138(24)**: 7568-7573.

Irmak, S., Kang, J. & Wilkins, M. (2020). Depolymerization of lignin by wet air oxidation. *Bioresource Technology Reports*. **9**: 100377.

Knesl, P., Röseling, D. & Jordis, U. (2006). Improved synthesis of substituted 6, 7-dihydroxy-4-quinazolineamines: tandutinib, erlotinib and gefitinib. *Molecules*. **11(4)**: 286-297.

Kumar, N., Chowdhary, A., Gudaparthi, O., Patel, N. G., Soni, S. K. & Sharma, P. (2014). A simple and highly efficient process for synthesis of Gefitinib and its intermediate *Indian Journal of Chemistry - Section B*. **53(10)**:1269-1274

Li, F., Feng, Y., Meng, Q., Li, W., Li, Z., Wang, Q. & Tao, F. (2007). An efficient construction of quinazolin-4 (3H)-ones under microwave irradiation. *Arkivoc*. **1**: 40-50.

Marzaro, G., Guiotto, A., Pastorini, G. & Chilin, A. (2010). A novel approach to quinazolin-4 (3H)-one via quinazoline oxidation: an improved synthesis of 4-anilinoquinazolines. *Tetrahedron*. **66(4)**: 962-968.

Nishino, S., Hiromitsu, K., Shima, Hidetaka & Harada, T., Oda, H. (2013). *Process for producing quinazolin-4-one derivative*. US 8367824.

Rakshit, S., Lakshminarasimhan, T., Guturi, S., Kanagavel, K., Kanusu, U.R., Niyogi, A.G., Sidar, S., Luzung, M.R., Schmidt, M.A., Zheng, B., Eastgate, M.D. & Vaidyanathan, R. (2018). Nitration using fuming HNO_3 in sulfolane: synthesis of 6-nitrovanillin in flow mode. *Organic Process Research & Development*. **22(3)**: 391-398.

Rewcastle, G.W. & Showalter, H. D. H. (2000). Synthesis of 4-(Phenylamino) pyrimidine derivatives as ATP-Competitive protein kinase inhibitors with potential for cancer chemotherap. *Current Organic Chemistry*. **4(7)**: 679-706.

Shaik, A., Bhagwat, P.U., Palanisamy, P., Chhabria, D., Dubey, P., Kirubakaran, S. & Thiruvenkatam, V. (2023). Novel pharmaceutical co-crystals of gefitinib: synthesis, dissolution, cytotoxicity, and theoretical studies. *CrystEngComm*. **25(17)**: 2570-2581.

Shan, Y., Liu, F., Ma, Y., Fan, T., Wang, M. & Dong, Y. (2016). Design, synthesis and biological evaluation of 2-Phenoxy-N-Phenylacetamides as novel anticancer agents. pharmaceutical chemistry journal. **49(12)**: 798-803.

Shih, K.S., Hsieh, Y.J. & Liu, C.W. (2011). *Synthetic method for 6,7-substituents-4-aniline quinazoline*. WO 2011147102

Silva, E.S., Marchi, R.C., Matos, C.S.P., Silva, M.F.G., Fernandes, J.B., Bueno, O.C. & Carlos, R.M. (2021). Insecticidal and fungicidal activity of a magnesium compound containing isovanillic acid against leaf-cutting ant and its symbiotic fungus. *Química Nova*. **44**: 267-271.