Jurnal Riset Kimia

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

Regioselective Synthesis of 5-Nitronaphtalene-1,4-dione Derivatives in Various Solvents and Their Characterization

Iin Narwanti[1*], Warsi Warsi[1], Bidyadhar Sethy[2]
[1]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[2]Taipei Medical University, Taipei City, Taiwan

Corresponding Author:
Iin Narwanti
iin.narwanti@pharm.uad.ac.id

Received: April 2025
Accepted: March 2026
Published: March 2026

© Iin Narwanti et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Regioselective synthesis is a crucial strategy in the development of bioactive molecules, as regioisomers may exhibit distinctly different biological activities. Heterocyclic naphthoquinones are a promising class of bioactive scaffolds with potential anticancer properties that might be influenced by their regiochemical configuration. This study aimed to investigate the regioselective synthesis of 5-nitronaphthalene-1,4-dione with an amine using various solvents and to characterize the target compounds. The synthesis was initiated with the nitration of 2,3-dichloronaphthalene-1,4-dione using a mixture of HNO_3/H_2SO_4, generating compound **2** in a moderate yield. Next, compound **2** was subsequently reacted with N, N-dimethylethane-1,2-diamine under alkaline conditions at ambient temperature, affording two regioisomeric target compounds (compounds **4** and **5**). The characterization confirmed the successful synthesis of compounds **4** and **5**, with findings indicating that aprotic solvents facilitated the formation of compound **4**, while protic solvents slightly favored the formation of compound **5**. In conclusion, the results reveal that the solvent governs regioselective synthesis of 5-nitronaphthalene-1,4-dione derivatives, providing a basis for controlling substitution patterns in these scaffolds. Further, the successful synthesis and isolation of regioisomerically pure compounds establishes a foundation for further exploration of their biological activities.

Keywords: *Regioselectivity, synthesis, 5-nitronaphthalene-1,4-dione, solvents*.

Introduction

Heterocyclic naphthoquinones are noteworthy for their extensive range of biological activities, including antiproliferative activities [1, 2], anticancer [3-5], antibacterial [6, 7], antifungal [6], antimalarial [8], and anti-inflammatory [9]. Their unique electrochemical properties also place naphthoquinones as promising structural scaffolds for innovative bio-electrochemical applications [10-12]. Recent studies have successfully synthesized various derivatives of 1,4-naphthoquinone. For example, Ravichandran and his team developed a series of 1,4-naphthoquinone derivatives linked to aryl/aliphatic amide and S-cyclohexane moieties. Their assessment of these compounds revealed moderate antibacterial activity [13]. Furthermore, Prachayasittikul and his team focused on synthesizing 2-amino-1,4-naphthoquinone compounds and subsequently assessed their effectiveness against many types of cancer cell lines. Notably, their findings indicated that the most active compound demonstrated remarkable efficacy against the T-lymphoblastic leukemia cell line, achieving an IC_{50} value of 2.118 μM [3]. These results not only highlight the therapeutic potential of naphthoquinone derivatives but also pave the

way for further exploration in both antibacterial and anticancer therapies.

The nitro group (NO_2) is a powerful electron-withdrawing group (EWG) with strong electron-attracting properties. Recognized as a privileged functional group [14], it has attracted significant attention in medicinal chemistry. Its significant electron-withdrawing characteristics play a crucial role in influencing the electronic properties as well as polarity of resulting molecules, leading to impactful interactions with certain amino acids (AAs) in target proteins [15]. When paired with aryl or heteroaryl structures, the nitro group showcases a wide array of biological activities, including potent anticancer, antitubercular, and antiparasitic effects. For instance, the nitro group attached to the quinone scaffold is known to enhance biological activity significantly.

Previous studies showed that substituted 2,3-dichloro-1,4-naphthoquinone can readily react with amines in alkaline conditions, yielding a mixture of two regioisomers (2- and 3-regioisomers) [16-18]. Commonly, some bases such as triethylamine (TEA), N, N-diisopropylethylamine (DIPEA), NaOH, KOH, K_2CO_3, and Cs_2CO_3 are used in this reaction. However, the regioselective synthesis of 5-nitro-naphthoquinone derivatives, specifically the reaction of 2,3-dichloro-5-nitronaphthalene-1,4-dione with N, N-dimethylethane-1,2-diamine, has not been previously reported. Regioisomeric products have different structural arrangements that can affect how they bind to biological targets, leading to different pharmacological activities. Controlling which regioisomer is formed is therefore essential for establishing structure–activity relationships and improving therapeutic potential. Therefore, this study addresses this gap by showing how solvent choice governs the regiochemical outcome of the reaction, providing a synthetic strategy for the selective preparation of specific regioisomeric 2,3-dichloro-5-nitronaphthalene-1,4-dione derivatives. Here, we investigated the solvent-controlled regioselective reaction of 2,3-dichloro-5-nitronaphthalene-1,4-dione with N, N-dimethylethane-1,2-diamine across protic and aprotic solvents, explicitly demonstrating that solvent polarity directs regioisomeric product formation in a nitro-substituted naphthoquinone system. Both regioisomers were comprehensively characterized by TLC, ^{1}H-NMR, ^{13}C-NMR, LC-MS, and their purity was confirmed by HPLC.

Experimental

Materials

All chemicals utilized in this research were of analytical or reagent grade, ensuring high quality and reliability. The specific compounds included 2,3-dichloronaphthalene-1,4-dione, N, N-dimethylethane-1,2-diamine, tetrahydrofuran (THF), dioxane, dichloromethane (DCM), methanol, ethanol, silica gels (Merck, 230~400 mesh), magnesium sulfate ($MgSO_4$), ethyl acetate, *n*-hexane, nitric acid (HNO_3), sulfuric acid (H_2SO_4), and diisopropylethylamine (DIPEA).

Instruments

The instruments employed in this research was as follows: analytical weight balance (ATY224 SHIMADZU, Uni Bloc), magnetic stirrer and hot plate (CORNING PC-420D), LC-MS (LCMS-2020, SHIMADZU), auto-sampler (LC-2030C LT, Prominence-I, SHIMADZU), melting point apparatus (Fargo MP-2D), UVGL-25 UV light, Bruker DRX-500 spectrometer, Zorbax Eclipse XDB-C18 Agilent column, TLC (ALUGRAM® Xtra SIL G/UV254, Buchi rotary evaporator, glassware.

Methods

Synthesis of compounds 2,3-dichloro-5-nitronaphthalene-1,4-dione (2) and 2,3-dichloro-6-nitronaphthalene-1,4-dione (3)

Compounds **2** and **3** were afforded following the protocol reported in the literature [19]. 2,3-Dichloronaphthalene-1,4-dione 1 (2.5 g, 0.011 mol) was gradually poured into a H_2SO_4 (25 g) and HNO_3 (7.5 g) mixture, and further was heated at 70°C. After stirring continuously for three hours, the color of the mixture turned into a dark-brown solution. Subsequently, it was

poured into ice water, resulting in a yellow solid precipitate. It was filtered off and washed with H_2O. The residue was isolated and purified by silica column chromatography (SCC) (*n*-hexane: ethyl acetate = 4:1) to give compounds **2** and **3**. The obtained yield of compounds **2** and **3** was 70% and 28%, respectively. Further, to confirm the structure of compounds **2** and **3**, NMR analysis was performed. And here is the spectral data of compounds **2** and **3**: compound **2**: ^{1}H-NMR (300 MHz, MeOD) δ_H 8.39 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.03 (dd, *J* = 7.7 Hz, 1H), 7.96 (dd, *J* = 8.0, 1.5 Hz, 1H); compound **3**: ^{1}H-NMR (300 MHz, MeOD) δH 8.88 (d, *J* = 2.3 Hz, 1H), 8.65 (dd, *J* = 8.5, 2.3 Hz, 1H), 8.40 (d, *J* = 8.5 Hz, 1H). Only compound **2** was further used for the preparation of compounds **4** and **5**.

Synthesis of compounds 2-chloro-3-((2-(dimethylamino)ethyl)amino)-5-nitronaphthalene-1,4-dione (4) and 3-chloro-2-((2-(dimethylamino)ethyl)amino)-5-nitronaphthalene-1,4-dione (5)

To a solution of compound **2** (136 mg, 0.5 mmol) in various solvents (toluene, dioxane, THF, dichloromethane, methanol, and ethanol (5.0 mL)) was added N, N-dimethylethane-1,2-diamine (82 µL, 0.75 mmol) and DIPEA (131 µL, 0.75 mmol). The solvents were selected to cover a wide range of polarity and hydrogen-bonding ability, allowing systematic investigation of how these properties influence regioisomer formation. The mixture was mixed and stirred at ambient temperature (25 ± 2°C) for 2 hours under an N_2 atmosphere. TLC was performed to check and monitor the completion of the reaction. The reaction mixture was added with H_2O, then extracted with DCM two times. The organic (DCM) layer was combined, followed by drying with anhydrous $MgSO_4$, filtered, and concentrated by a rotary evaporator to get the crude target compound. The resulting crude target compound was purified by using SCC by eluting with *n*-hexane: ethyl acetate: MeOH = 3:2:0.1 to obtain compounds **4** and **5**.

Characterization of the compounds 2-chloro-3-((2-(dimethylamino)ethyl)amino)-5-nitronaphthalene-1,4-dione (4) and 3-chloro-2-((2-(dimethylamino)ethyl)amino)-5-nitronaphthalene-1,4-dione (5)

Compounds **4** and **5** were further characterized to determine their structure. Melting points (mp) of these two compounds were determined utilizing a Fargo MP-2D melting point apparatus, which allows for measurement of melting temperatures to qualitatively assess purity and compound identity. The nuclear magnetic resonance (NMR) spectra, specifically ^{1}H-NMR and ^{13}C-NMR, were recorded on a Bruker DRX-500 spectrometer, operating at frequencies of 300 MHz and 75 MHz, respectively. For NMR analysis, tetramethylsilane (TMS) was adopted as the internal standard to calibrate chemical shifts, with deuterated solvents, either $CDCl_3$ (deuterated chloroform) or MeOD (deuterated methanol), employed to enhance spectral clarity. Chemical shifts (denoted as δ) were quantified in parts per million (ppm), while coupling constants (denoted as *J*) were reported in Hertz (Hz). In the case of ^{1}H-NMR, chemical shifts were calibrated to the residual solvent peaks at 3.31 ppm for MeOD and 7.26 ppm; for ^{13}C-NMR, the corresponding calibration was to 49.00 ppm for MeOD and 77.00 ppm for $CDCl_3$. The isolation of final compounds was achieved through SCC, which ensures effective separation based on compound polarity. TLC was performed on silica gel plates to monitor reaction progress and compound purity, with spot visualization under UV light at 254 nm. LC-MS spectra were obtained using a Shimadzu LC2020 instrument, allowing for the determination of molecular weights and structural insights into the compounds. The purity of the isolated compounds was assessed using an Agilent system, where a mobile phase composed of acetonitrile and water, comprising 10 mM ammonium acetate and 0.1% (w/w) formic acid (HCOOH), was utilized. The separation was conducted on a Zorbax Eclipse XDB-C18 Agilent column (particle size = 5 µm, dimensions = 150 mm x 4.6 mm), ensuring high-

DOI: https://doi.org/10.25077/jrk.v17i1.837

resolution separation and accurate determination of compound purity.

Results and Discussion

To successfully synthesize the desired target compounds, the initial preparation of compound **2** was a crucial step. The synthetic scheme outlining this intermediate's formation is illustrated in **Figure 1**. Utilizing the method established by Stoddard and the team [19], we treated 2,3-dichloronaphthalene-1,4-dione with a concentrated mixture of HNO_3 and H_2SO_4. This reaction yielded two regioisomers, showcasing the complexity of the process. As noted by Gulsah and the team [20], the reaction produced 2,3-dichloro-5-nitronaphthalene-1,4-dione (compound **2**) and 2,3-dichloro-6-nitronaphthalene-1,4-dione (compound **3**). To effectively separate these isomers, we employed column chromatography using a 4:1 *n*-hexane-ethyl acetate solvent mixture. This separation not only yielded pure compounds but also enabled us to confirm their structures through detailed NMR analysis. After isolating compound **2**, we treated it with N, N-dimethylethane-1,2-diamine under alkaline conditions at room temperature (**Figure 2**). This step involved the careful selection of various protic and aprotic solvents, which facilitated the successful transformation into our target compounds.

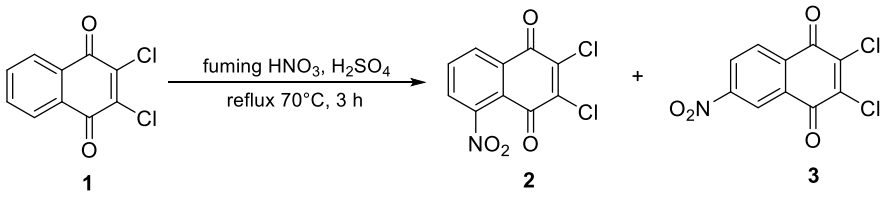


Figure 1. The synthetic approach of compounds **2** and **3**.

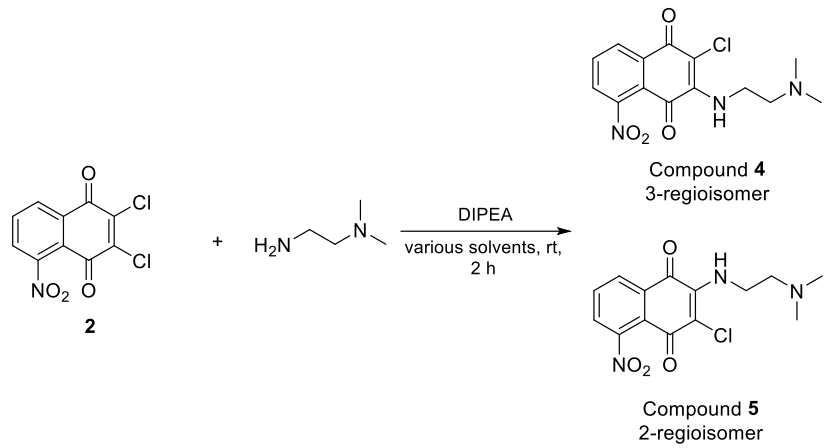


Figure 2. The synthetic approach of compounds **4** and **5.**

As depicted in **Figure 2**, the reaction of the compound **2** with an amine under alkaline conditions afforded the formation of two distinct regioisomers: 3-regioisomer (compound **4**) and 2-regioisomer (compound **5**). The reaction of 2,3-dichloro-5-nitronaphthalene-1,4-dione with amines is a nucleophilic aromatic substitution (SNAr) process. Especially, it is an addition-elimination process where the amine acts as the nucleophile, targeting the electrophilic carbon atom at the C2 or C3 positions of the quinone ring. The strongly electron-withdrawing NO_2 group availability at C5, combined with the two carbonyl groups (C1=O and C4=O) of the quinone, makes the chlorine atoms highly susceptible to displacement by nucleophiles such as amines. Firstly, nucleophilic addition occurs when the amine attacks one of the carbon

atoms bearing a chlorine atom (at C2 or C3). Due to the asymmetry introduced by the 5-NO_2 group, the electron density is nonuniform, leading to regioselective substitution and often producing a mixture of 3-amino-2-chloro-5-nitro (3-regioisomer or compound **4**) and 2-amino-3-chloro-5-nitro (2-regioisomer or compound **5**). Secondly, elimination of the leaving (Cl^-) group happens by the reformation of the double bond upon negatively charged oxygen of the carbonyl group resonates towards the quinone ring, leading to the expulsion of the chloride ion (Cl^-). Lastly, an excess equivalent of amine or an external base like DIPEA removes a proton from the newly attached amino group to neutralize and get the desired product (**Figure 3**).

To separate the two regioisomers, column chromatography was employed using an isocratic eluent of *n*-hexane, ethyl acetate, and MeOH (3:2:0.1, v/v/v). This separation method proved to be advantageous, as it facilitated a thorough examination of their individual physical properties. Key characteristics such as TLC, color, and physical state, melting point, and retention factor (Rf) were analyzed to distinguish the compounds further. The TLC profile, comparing the product to the starting material, is shown in **Figure 4** and reveals crucial differences.

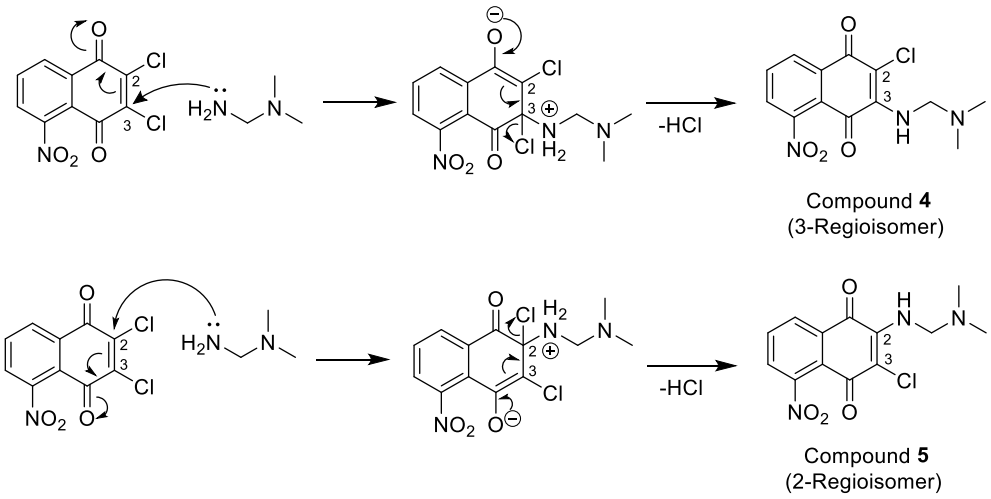


Figure 3. The proposed reaction mechanism



Figure 4. TLC profile of compounds **4** and **5** compared to starting material (*n*-hexane: ethyl acetate: MeOH = 3:2:0.1 as mobile phase, A = 2,3-dichloro-5-nitronaphthalene-1,4-dione 2, B = compound **4**, C = compound **5**, D = co- compounds **4** and **5**, E = = co-2,3-dichloro-5-nitronaphthalene-1,4-dione, compounds **4** and **5**, detection = UV_{254})

DOI: https://doi.org/10.25077/jrk.v17i1.837

Table 1. The color, melting point, and retention factor (R_f) of compounds **4** and **5**

Compound	Color and physical state	Melting Point (°C)	Retention Factor (R_f)[a]
4	Orange solid	145-146	0.26
5	Dark red solid	155-156	0.08

[a]The retention factor (R_f) values were determined by developing the TLC plate twice using *n*-hexane: ethyl acetate: methanol (3:2:0.1, v/v/v) as the eluent

The R_f values indicated that compound **4** had slightly higher mobility than compound **5** in a normal phase system. Specifically, the R_f values were measured at 0.26 for compound **4** and 0.08 for compound **5** when developed twice using a solvent mixture of *n*-hexane, ethyl acetate, and MeOH (ratio of 3:2:0.1). In addition to the TLC analysis, a summary of the physical properties of compounds **4** and **5** is presented in **Table 1**. Notably, these two regioisomers are differentiated not only by their distinct melting points, 145-146°C for compound **4** and 155-156°C for compound **5**, but also by their varying colors. These observations underscore the unique properties of each regioisomer and provide valuable insights for future research and potential applications involving these compounds.

An analysis of the regioselectivity in the reaction of 2,3-dichloro-5-nitronaphthalene with N, N-dimethylethane-1,2-diamine, utilizing DIPEA as a base across various solvents, is shown in **Table 2**. The results indicate that the solvent plays a pivotal role in influencing the reaction outcomes. In aprotic solvents such as toluene, THF (tetrahydrofuran), dioxane, and dichloromethane (DCM), the formation of compound **4** was significantly favored. In comparison, protic solvents, including methanol and ethanol, demonstrate a slight preference for the formation of compound **5**. Although the precise mechanisms underlying these observations remain to be fully elucidated, the findings are consistent with those reported by Yoon and team [21]. Their study examined the regioselectivity of 6,7-dihaloquinoline-5,8-diones reacting with amine nucleophiles in various solvents, underscoring the vital role that solvent choice plays in determining reaction pathways. This reinforces the importance of considering solvent effects in future research endeavors within this field.

The regioselectivity observed is strongly governed by solvent effects, particularly dielectric constant, polarity, and protic–aprotic character (**Table 2**). In nonpolar and weakly polar aprotic solvents (toluene and 1,4-dioxane), compound **4** predominates, indicating that low dielectric environments favor pathways involving less polar transition states and minimal solvation of the nucleophile. The regioselectivity of the reaction strongly favors the 3-regioisomer (compound **4**) in nonpolar to moderately polar aprotic solvents, with the compound **4**/**5** ratio dropping progressively from 78/22 in toluene (ε = 2.38) to 51/49 in DCM (ε = 8.93) as the dielectric constant increases, demonstrating a clear inverse correlation between solvent polarity and selectivity for compound **4**. As solvent polarity increases (THF and dichloromethane), selectivity decreases, reflecting enhanced stabilization of polar intermediates and the emergence of competing pathways. This trend becomes more pronounced in polar protic solvents, where methanol (ε = 32.6) and ethanol (ε = 24.3) both reverse the selectivity in favor of the 2-regioisomer (compound **5**), giving ratios of 44/56 and 47/53, respectively, likely due to hydrogen-bonding interactions and stronger solvation of charged species, which modify nucleophile reactivity and stabilize alternative transition states. Such behavior is consistent with established solvent effects in nucleophilic

aromatic substitution (SNAr) reactions, where solvent polarity and hydrogen-bonding ability play key roles in stabilizing the intermediates and controlling regioselectivity [22].

The proton and carbon spectra of compounds **4** and **5** are presented in **Figure 5** and are summarized in **Table 3**. Overall, the multiplicity and splitting patterns of both compound spectra are quite similar. A broad singlet at δ 6.94 ppm (1H) is likely attributed to the -NH group. A quartet at δ 3.89 and 3.90 ppm (J = 5.5 Hz, 2H) associated with a methylene (-CH_2) adjacent to the -NH group, while the triplet at δ 2.60 and 2.57 ppm (J = 5.9 Hz, 2H) indicates another methylene group. A singlet at δ 2.31 and 2.28 ppm, respectively, integrating for 6H, suggests the presence of two equivalent methyl groups in a symmetric environment. Overall, the multiplicities and coupling constants indicate a structure composed of a substituted aromatic ring, a methylene chain, and methyl groups. The main difference of these compounds lies in the chemical shift of the protons in the aromatic ring.

The ^{1}H-NMR spectra of the synthesized two regioisomers indicated that the peaks of compound **4** of aromatic protons are shifted more downfield (deshielded) than the aromatic protons of compound **5** (shielded). The regiochemistry of the two isomers can be unambiguously distinguished by monitoring the chemical shift changes of H6 and H7 in the ^{1}H-NMR spectrum (**Figure 6**). In compound **4** (3-regioisomer) the amine substituent is located at C3, adjacent to C4═O, which places it farther from H6 but transmits a deshielding influence through the conjugated quinone framework, resulting in H6 and H7 appearing at comparatively downfield positions. In contrast, in compound **5** (2-regioisomer), the amine at C2 is directly conjugated through C1═O toward the benzenoid ring, effectively donating electron density through resonance to H7 and shifting it to a noticeably upfield position relative to compound **4**, while H6 remains largely downfield in both isomers due to the persistent deshielding effect of the fixed C5-nitro group.

Table 2. Regioselectivity in the reaction of 2,3-dichloro-5-nitronaphthalene-1,4-dione and N, N-dimethylethane-1,2-diamine in various solvents

Solvent	Dielectric Constant (ε)[a]	Category	Type	Yield (%)[b]		Ratio (4/5)
				Compound 4	Compound 5	
Toluene	2.38	Nonpolar	Aprotic	73	21	78/22
1,4-Dioxane	2.21	Weakly polar	Aprotic	72	24	75/25
THF	7.58	Moderately polar	Aprotic	65	31	68/32
DCM	8.93	Moderately polar	Aprotic	49	47	51/49
Methanol	32.6	Polar	Protic	42	54	44/56
Ethanol	24.3	Polar	Protic	47	52	47/53

[a]Dielectric Constant (ε), at 25^{0}C, was retrieved from the literature[23]

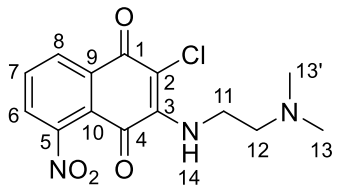
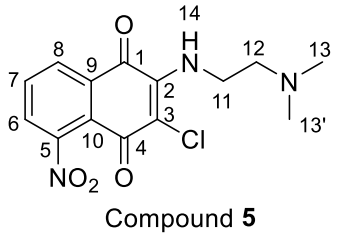
[b]Isolated by silica column chromatography, *n*-hexane: ethyl acetate: MeOH = 3:2:0.1 as mobile phase

DOI: https://doi.org/10.25077/jrk.v17i1.837

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

Therefore, the downfield shift of H7 in compound **4** versus its upfield shift in compound **5**, combined with the relatively invariant position H6, serves as a definitive spectroscopic fingerprint to systematically assign the amine substitution position and distinguish the two regioisomers. This result is in line with the reported literature, which also successfully synthesized and determined 5-nitro-naphthoquinone derivatives, followed by structural confirmation using the X-ray crystallography method [24].

Table 3. Summarized spectral data of compounds **4** and **5**

Structure	Spectral data[a]
 Compound **4**	^{1}H-NMR (300 MHz, Chloroform-*d*) δH 8.34 (dd, J = 7.8, 1.2 Hz, 1H, H6), 7.83 (dd, J = 7.8, 8.1 Hz, 1H, H7), 7.61 (dd, J = 7.9, 1.2 Hz, 1H, H8), 6.94 (br s, 1H, NH), 3.89 (q, J = 5.5 Hz, 2H, H11), 2.60 (t, J = 5.9 Hz, 2H, H12), 2.31 (s, 6H, H13 and H13′); ^{13}C-NMR (125 MHz, Chloroform-*d*) δH 177.4 (s, C4), 174.2 (s, C1), 148.4 (s, C5), 145.0 (s, C3), 135.2 (d, C7), 133.6 (s, C10), 129.0 (d, C8 and C9), 126.0 (d, C6), 121.0 (s, C2), 57.6 (t, C12), 44.8 (q, C13 and C13′), 42.0 (t, C11); MS (ESI)[a] = 324.05 $[M+H]^+$.
 Compound **5**	^{1}H-NMR (300 MHz, Chloroform-*d*) δH 8.19 (dd, J = 7.7, 1.4 Hz, 1H, H6), 7.73 (dd, J = 7.8, 7.8 Hz, 1H, H7), 7.63 (dd, J = 8.0, 1.3 Hz, 1H, H8), 6.94 (br s, 1H, NH), 3.90 (q, J = 5.6 Hz, 2H, H11), 2.57 (t, J = 5.9 Hz, 2H, H12), 2.28 (s, 6H, H13 and H13′); ^{13}C-NMR (151 MHz, DMSO-*d6*) δH 177.3 (s, C4), 171.7 (s, C1), 147.7 (s, C5), 133.8 (d, C7 and C9), 131.9 (s, C2), 128.5 (d, C8), 127.8 (d, C6), 122.3 (s, C10), 108.3 (s, C3), 58.5 (t, C12), 44.5 (q, C13 and C13′), 41.8 (t, C11); MS (ESI)[a] = 324.05 $[M+H]^+$.

[a]The MS spectra of compounds **4** and **5** were attached in the supplementary file

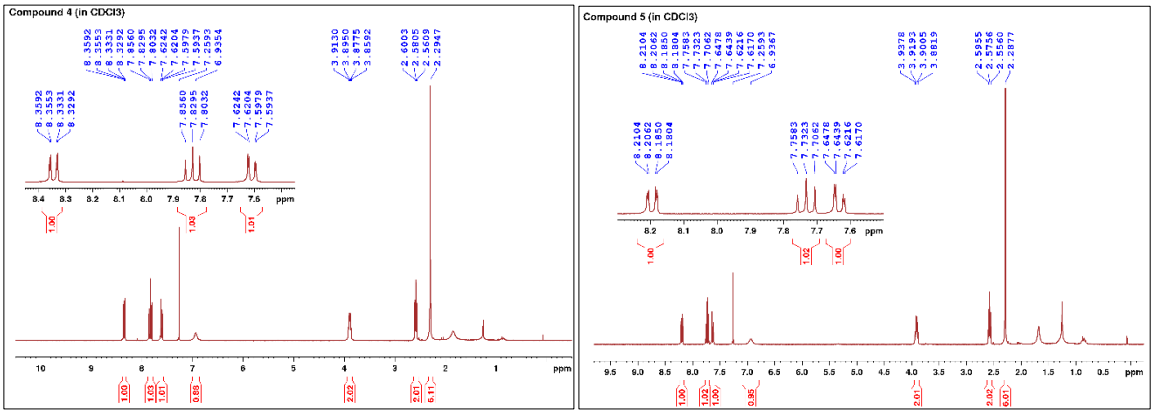


Figure 5. ^{1}H-NMR spectra of compounds **4** and **5**

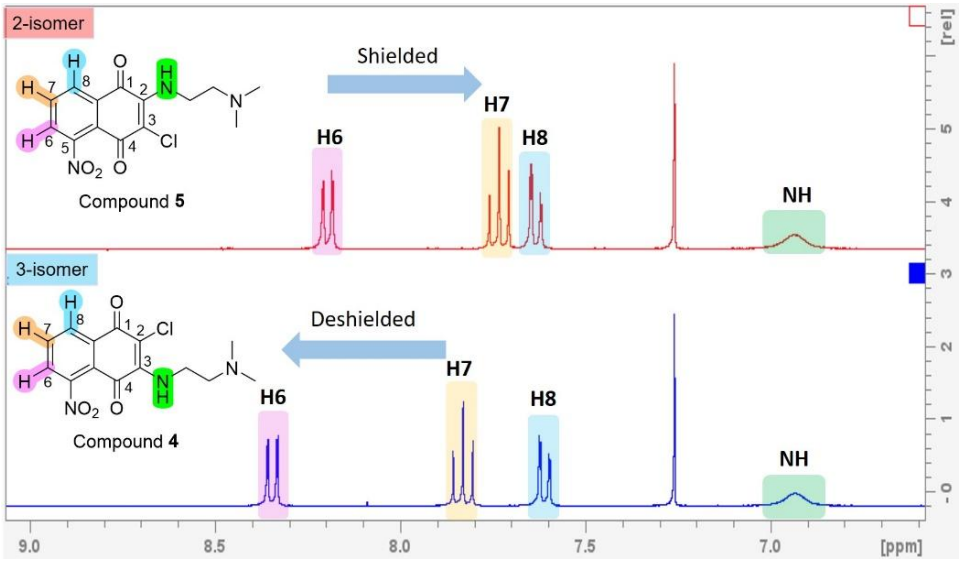


Figure 6. ^{1}H-NMR spectra comparison of compounds **4** and **5**

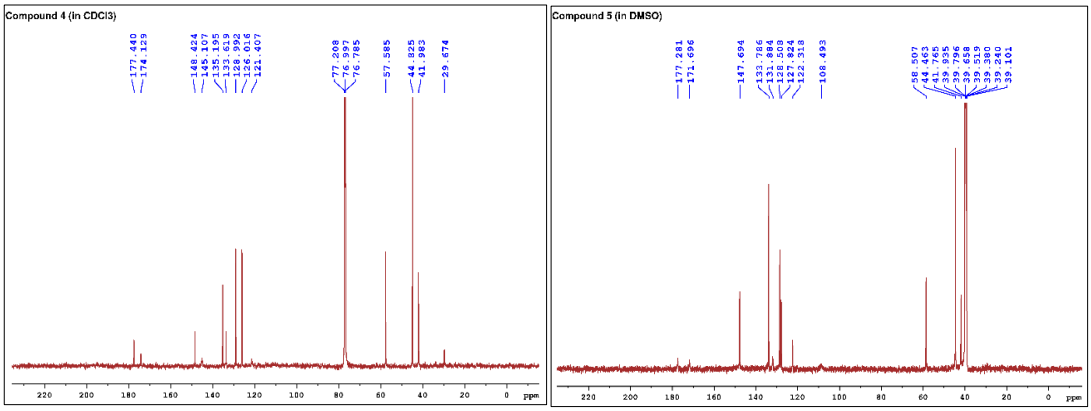


Figure 7. ^{13}C-NMR spectra of compounds **4** and **5**

The ^{13}C-NMR (**Figure 7**) spectra of the two regioisomers suggested that some peaks are broader and less visible. This is due to the tendency of quinolinequinone compounds to tautomerize [25]. Therefore, as a consequence of this reason, the ^{13}C-NMR spectra of C1, C2, and C3 are broader and less visible for compound **4** with chemical shift (δ) of 174.2, 121.0, and 145.0 ppm, respectively. While C1, C2, C3, and C4 are less visible for compound **5**, with chemical shift (δ) of 171.7, 108.3, and 131.9, respectively.

Compounds **4** and **5** were further characterized by HPLC to determine their purity, following the conditions as defined in the experimental section. Both Compounds **4** and **5** contain a notable peak at the same retention time of 11.077 and 10.974 min, respectively, in their HPLC chromatograms (**Figure 8**), suggesting they are structurally similar compounds. Minor peaks are observed in both spectra, but they are modest and well-defined, indicating that contaminants are negligible. The overall HPLC profile indicates that both compounds are most likely pure (purity > 95%), with efficient separation and a consistent retention time under the current conditions.

Despite the study showing promising results, it has some limitations, including the absence of X-ray crystal structure confirmation. As a future direction, following the successful separation of the two regioisomers of 5-nitronaphthalene-1,4-diones derivatives, investigation of their biological activities, particularly as antibacterial and anticancer agents, becomes feasible for future studies.

DOI: https://doi.org/10.25077/jrk.v17i1.837

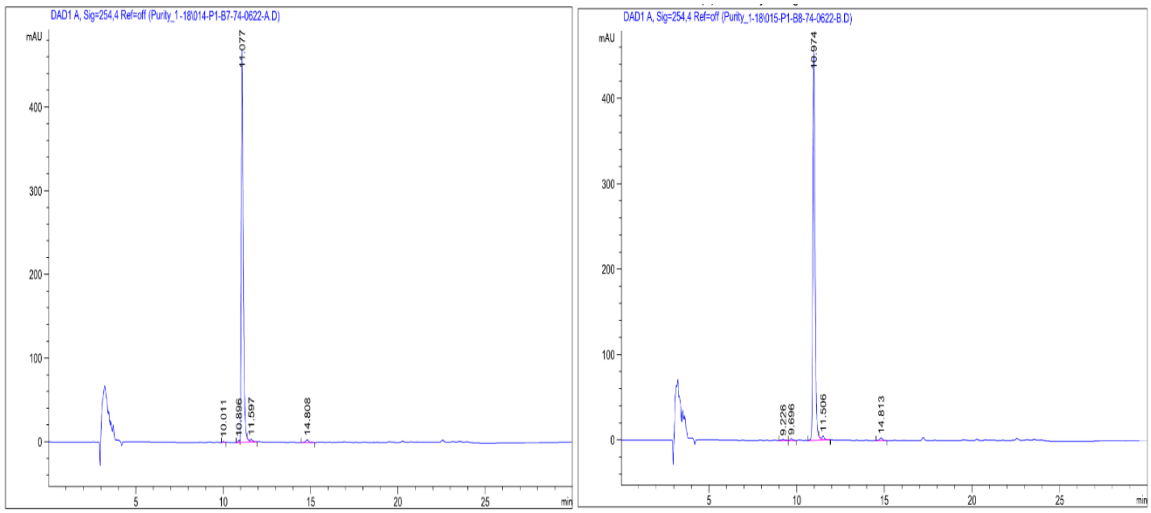


Figure 8. HPLC profile of compounds **4** and **5**

Conclusions

Regioselective synthesis of 5-nitronaphthalene-1,4-dione derivatives in various protic and aprotic solvents was successfully performed using 2,3-dichloro-5-nitronaphthalene-1,4-dione and amine under mild conditions, generating a satisfactory cumulative yield (>90%). The solvent used plays a crucial role in influencing the formation of each regioisomer. We found that compound **4** formation was substantially more favorable in aprotic solvents, but compound **5** formation increased modestly in a protic solvent. The TLC profile showed that compound **4** was slightly more non-polar than compound **5**. The spectral data confirmed the structure of the expected 5-nitronaphthalene-1,4-diones.

Acknowledgment

This research was supported by LPPM Universitas Ahmad Dahlan.

Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

W.W.: Methodology, investigation, data collection, formal analysis, data curation, visualization, and writing —original draft preparation. B.S.: Methodology, validation, and writing —review and editing. I.N.: Conceptualization, corresponding author responsibilitiesand, and supervision. Both authors have read and approved the final version of the manuscript.

Funding

This research was funded by LPPM Universitas Ahmad Dahlan.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

References

1. S. Uysal, Z. Soyer, M. Saylam, A.H. Tarikogullari, S. Yilmaz, P.B. Kirmizibayrak, Design, synthesis and biological evaluation of novel naphthoquinone-4-aminobenzensulfonamide/carboxamide derivatives as proteasome inhibitors, *European Journal of Medicinal Chemistry*, **209**, 112890, (2021), https://doi.org/10.1016/j.ejmech.2020.112890
2. I.A. Schepetkin, A.S. Karpenko, A.I.

Khlebnikov, M.O. Shibinska, I.A. Levandovskiy, L.N. Kirpotina, N.V. Danilenko, M.T. Quinn, Synthesis, anticancer activity, and molecular modeling of 1,4-naphthoquinones that inhibit MKK7 and Cdc25, *European Journal of Medicinal Chemistry*, **183**, 111719, (2019), https://doi.org/10.1016/j.ejmech.2019.111719

3. V. Prachayasittikul, R. Pingaew, A. Worachartcheewan, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Synthesis, anticancer activity and QSAR study of 1,4-naphthoquinone derivatives, *European Journal of Medicinal Chemistry*, **84**, 247-263, (2014), https://doi.org/10.1016/j.ejmech.2014.07.024

4. D. Bhasin, S.N. Chettiar, J.P. Etter, M. Mok, P.-K. Li, Anticancer activity and SAR studies of substituted 1,4-naphthoquinones, *Bioorganic & Medicinal Chemistry*, **21**, 4662-4669, (2013), https://doi.org/10.1016/j.bmc.2013.05.017

5. T. Qin, Y.-Y. Ma, C.-E. Dong, W.-L. Wu, Y.-Y. Feng, S. Yang, J.-B. Su, X.-X. Si, X.-J. Wang, D.-H. Shi, Design, synthesis, cytotoxicity evaluation and molecular docking studies of 1,4-naphthoquinone derivatives, *Journal of Molecular Structure*, **1263**, 133067, (2022), https://doi.org/10.1016/j.molstruc.2022.133067

6. V.K. Tandon, H.K. Maurya, N.N. Mishra, P.K. Shukla, Design, synthesis and biological evaluation of novel nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents, *European Journal of Medicinal Chemistry*, **44**, 3130-3137, (2009), https://doi.org/10.1016/j.ejmech.2009.03.006

7. M. Janeczko, O.M. Demchuk, D. Strzelecka, K. Kubiński, M. Masłyk, New family of antimicrobial agents derived from 1,4-naphthoquinone, *European Journal of Medicinal Chemistry*, **124**, 1019-1025, (2016), https://doi.org/10.1016/j.ejmech.2016.10.034

8. G.C. Brandão, F.C. Rocha Missias, L.M. Arantes, L.F. Soares, K.K. Roy, R.J. Doerksen, A. Braga de Oliveira, G.R. Pereira, Antimalarial naphthoquinones. Synthesis via click chemistry, in vitro activity, docking to PfDHODH and SAR of lapachol-based compounds, *European Journal of Medicinal Chemistry*, **145**, 191-205, (2018), https://doi.org/10.1016/j.ejmech.2017.12.051

9. R.X. Faria, F.H. Oliveira, J.P. Salles, A.S. Oliveira, N.L. von Ranke, M.L. Bello, C.R. Rodrigues, H.C. Castro, A.R. Louvis, D.L. Martins, V.F. Ferreira, 1,4-Naphthoquinones potently inhibiting P2X7 receptor activity, *European Journal of Medicinal Chemistry*, **143**, 1361-1372, (2018), https://doi.org/10.1016/j.ejmech.2017.10.033

10. Y.M. Hijji, B. Barare, Y. Zhang, Lawsone (2-hydroxy-1,4-naphthoquinone) as a sensitive cyanide and acetate sensor, *Sensors and Actuators B: Chemical*, **169**, 106-112, (2012), https://doi.org/10.1016/j.snb.2012.03.067

11. E.M. Sherif, S.-M. Park, Effects of 1,4-naphthoquinone on aluminum corrosion in 0.50M sodium chloride solutions, *Electrochimica Acta*, **51**, 1313-1321, (2006), https://doi.org/10.1016/j.electacta.2005.06.018

12. P. Ravichandiran, A. Boguszewska-Czubara, M. Masłyk, A.P. Bella, S.A. Subramaniyan, P.M. Johnson, K.S. Shim, H.G. Kim, D.J. Yoo, Naphthoquinone-based colorimetric and fluorometric dual-channel chemosensor for the detection of Fe2+ ion and its application in bio-imaging of live cells and zebrafish, *ACS Sustainable Chemistry & Engineering*, **7**, 17210-17219, (2019), https://doi.org/10.1021/acssuschemeng.9b03822

13. P. Ravichandiran, M. Masłyk, S. Sheet, M. Janeczko, D. Premnath, A. Kim, B. Park, M. Han, D. Yoo, Synthesis and antimicrobial evaluation of 1,4-naphthoquinone derivatives as potential antibacterial agents, *ChemistryOpen*, **8**, 589-600, (2019), https://doi.org/10.1002/open.201900077

 DOI: https://doi.org/10.25077/jrk.v17i1.837

14. K. Nepali, H.-Y. Lee, J.-P. Liou, Nitro-group-containing drugs, *Journal of Medicinal Chemistry*, **62**, 2851-2893, (2019), https://doi.org/10.1021/acs.jmedchem.8b00147

15. S. Noriega, J. Cardoso-Ortiz, A. Lopez-Luna, M.D.R. Cuevas-Flores, J.A. Flores De La Torre, The diverse biological activity of recently synthesized nitro compounds, *Pharmaceuticals (Basel)*, **15**, (2022), https://doi.org/10.3390/ph15060717

16. B.S. Samant, C. Chakaingesu, Novel naphthoquinone derivatives: Synthesis and activity against human African trypanosomiasis, *Bioorganic & Medicinal Chemistry Letters*, **23**, 1420-1423, (2013), https://doi.org/10.1016/j.bmcl.2012.12.075

17. C. Blackburn, Solid-phase synthesis of 2-amino-3-chloro-5- and 8-nitro-1,4-naphthoquinones: a new and general colorimetric test for resin-bound amines, *Tetrahedron Letters*, **46**, 1405-1409, (2005), https://doi.org/10.1016/j.tetlet.2005.01.049

18. Y. Li, A. Loureiro, M. Nguyen, M. Laurent, C. Bijani, F. Benoit-Vical, A. Robert, Y. Liu, B. Meunier, Synthesis and antimalarial activities of new hybrid atokel molecules, *ChemistryOpen*, **11**, e202200064, (2022), https://doi.org/10.1002/open.202200064

19. E.G. Stoddard, B.J. Killinger, R.N. Nair, N.C. Sadler, R.F. Volk, S.O. Purvine, A.K. Shukla, J.N. Smith, A.T. Wright, Activity-based probes for isoenzyme- and site-specific functional characterization of glutathione S-transferases, *Journal of the American Chemical Society*, **139**, 16032-16035, (2017), https://doi.org/10.1021/jacs.7b07378

20. N. Gulsah Deniz, A. F.Sh. Abdassalam, C. Sayil, O. Uguz, A. Koca, Regioselective synthesis of novel 5-nitro-naphthoquinone derivatives: Electrochemistry and in-situ spectroelectrochemistry properties, *Journal of Photochemistry and Photobiology A: Chemistry*, **431**, 114064, (2022), https://doi.org/10.1016/j.jphotochem.2022.114064

21. E.Y. Yoon, H.Y. Choi, K.J. Shin, K.H. Yoo, D.Y. Chi*, D.J. Kim, The regioselectivity in the reaction of 6,7-dihaloquinoline-5,8-diones with amine nucleophiles in various solvents, *Tetrahedron Letters*, **41**, 7475-7480, (2000), https://doi.org/10.1016/S0040-4039(00)01278-8

22. P. Campodonico, Solvent Effect on a Model of S_NAr Reaction in Conventional and Non-Conventional Solvents, in: D. Glossman-Mitnik, M. Maciejewska (Eds.) Solvents, Ionic Liquids and Solvent Effects, IntechOpen, London, 2019.

23. J. Rumble, T.J. Bruno, M.J. Doa, CRC Handbook of Chemistry and Physics, CRC Press, 2023.

24. I. Narwanti, Z.-Y. Yu, B. Sethy, M.-J. Lai, H.-Y. Lee, P. Olena, S.-B. Lee, J.-P. Liou, 6-Regioisomeric 5,8-quinolinediones as potent CDC25 inhibitors against colorectal cancers, *European Journal of Medicinal Chemistry*, **258**, 115505, (2023), https://doi.org/10.1016/j.ejmech.2023.115505

25. B.J. Mulchin, C.G. Newton, J.W. Baty, C.H. Grasso, W.J. Martin, M.C. Walton, E.M. Dangerfield, C.H. Plunkett, M.V. Berridge, J.L. Harper, M.S. Timmer, B.L. Stocker, The anti-cancer, anti-inflammatory and tuberculostatic activities of a series of 6,7-substituted-5,8-quinolinequinones, *Bioorganic & Medicinal Chemistry*, **18**, 3238-3251, (2010), https://doi.org/10.1016/j.bmc.2010.03.021