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Synthesis and characterization of 7-substituted 3-bromo-1, 4-dimethoxyphenazin-2(10h)-one derivatives

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Abstract

Phenazine-2(10H)-one derivatives represent a prominent class of nitrogen-containing tricyclic heterocycles that have attracted extensive research interest in medicinal chemistry due to their diverse pharmacological properties, including exceptional antimicrobial, antifungal, anti-inflammatory, and antiproliferative activities. In this study, a series of novel 7-substituted 3-bromo-1, 4-dimethoxyphenazin-2(10H)-one derivatives (6a-d) was efficiently synthesized through a streamlined multi-step chemical pathway. The key reactive building block, 2, 5-dibromo-3, 6-dimethoxy-1, 4-benzoquinone, was initially prepared via multi-step functionalization of hydroquinone involving oxidation, acid-catalyzed methylation, and subsequent regioselective bromination using N-bromosuccinimide. Condensation of this central quinone intermediate with various substituted *ortho*-phenylenediamines in the presence of anhydrous sodium acetate under refluxing ethanol successfully afforded the corresponding target phenazinone derivatives. The structures of all newly synthesized compounds were comprehensively verified and characterized using rigorous spectroscopic methods, including FTIR, ¹H NMR, ¹³C NMR, and ESI Mass Spectrometry. This synthesis provides a robust and scalable methodology for generating structurally diverse halogenated and alkylated dimethoxyphenazinone scaffolds, positioning them as promising structural templates for future targeted drug design and biomedical evaluations.

Keywords: Phenazine-2(10h)-one, hydroquinone, 1, 4-benzoquinone, analytical characterization.

Introduction

Developing novel heterocycles is a top modern priority to combat aggressive multidrug-resistant pathogens ^[1]. Among these frameworks, phenazin-2(10H)-one derivatives represent crucial nitrogen-containing tricyclic systems. These scaffolds display an outstanding array of crucial biological profiles ^[2, 3]. Phenazinone natural products act as highly effective redox-active templates in cellular systems ^[4]. They easily cross bacterial lipid bilayers to disrupt cellular respiration chains. This action imparts excellent antibacterial properties against stubborn Gram-positive clinical isolates ^[5, 6]. Additionally, these tricyclic matrices demonstrate significant broad-spectrum antifungal profiles against damaging agricultural crop molds ^[7]. Beyond antimicrobial capabilities, targeted functionalized phenazinones exhibit excellent antiproliferative activities. They trigger cell apoptosis by intercalating smoothly into human cancer cell lines ^[8, 9]. They also serve as highly potent enzyme inhibitors against critical intracellular targets. Specifically, they block topoisomerase pathways required for rapid malignant replication ^[10, 11]. Furthermore, recent trials confirm their outstanding anti-inflammatory capacities via cytokine mitigation ^[12]. They also display notable antimalarial capabilities by inducing fatal oxidative stress in intracellular parasites ^[13]. The profound pharmacological value of these molecules has driven diverse core construction methodologies. For instance, an innovative enzymatic synthesis was successfully achieved via oxidative cross-coupling. This laccase-catalyzed approach coupled simple hydroquinones with *ortho*-phenylenediamines cleanly via tandem Michael additions ^[14]. Alternatively, specialized tricyclic models were constructed by reacting heavily decorated benzoquinones. This sequence reacted substituted 1,4-benzoquinones with dimethoxy-*ortho*-phenylenediamines under thermal conditions ^[15, 16]. Further developments

Expanded these frameworks into complex fused ring structures. Reacting functionalized 2-hydroxy-1, 4-1, 4-flexible steps allow precise insertion of unique lipophilic fragments around the outer ring boundaries [26]. Building upon these valuable historical precedents, this work details a robust multi-step pathway to generate novel 7-substituted 3-bromo-1, 4-dimethoxyphenazin-2(10H)-one derivatives (6a-d). Combining highly electronegative bromine with electron-donating methoxy groups alters the core electronics. This deliberate spatial adjustment optimizes binding interactions within deep biological receptors. It also significantly increases lipophilicity to maximize cellular membrane permeability.

The current investigation outlines a streamlined synthesis of the key building block, 2, 5-dibromo-3, 6-dimethoxy-1, 4-benzoquinone, starting directly from commercial hydroquinone. This core intermediate undergoes subsequent base-assisted condensation with various substituted *ortho*-phenylenediamines to generate target small molecules. Every newly synthesized derivative is structurally confirmed using rigorous multi-nucleic NMR and mass spectrometry. This exploration offers a scalable synthetic platform to expand small-molecule libraries for targeted drug discovery campaigns.

Materials and methods: All solvents and reagents were

Purchased as laboratory-grade materials. Melting points were determined using the open capillary method and are reported uncorrected. Reaction monitoring for all novel products was carried out via thin-layer chromatography (TLC) utilizing Merck silica Gel-G plates. Analytical characterization was performed by recording FTIR spectra in KBr pellets on a Nexus 470 spectrometer, ^1H NMR spectra recorded in DMSO- d_6 solvent on Varian Mercury 400 MHz spectrometer using tetramethylsilane (TMS) as internal standard, ^{13}C NMR spectra recorded on Varian Mercury 75 MHz spectrometer and ESI-Mass spectra obtained on Shimadzu mass spectrometer.

Preparation of 2, 5-dibromo-3, 6-dimethoxy-1, 4-benzoquinone (4)

First, hydroquinone (1) was oxidized using hydrogen peroxide under alkaline conditions to produce 2, 5-dihydroxy-1, 4-benzoquinone (2) [27]. Next, the hydroxyl groups of this intermediate were protected by reacting it with methanol in an acidic environment, which yielded 2, 5-dimethoxy-1, 4-benzoquinone (3) [28]. This product was then brominated at room temperature using N-bromosuccinimide in dimethylformamide to generate 2, 5-dibromo-3, 6-dimethoxy-1, 4-benzoquinone (4) [29]. Finally, the chemical structure of compound (4) was verified through spectral analysis.

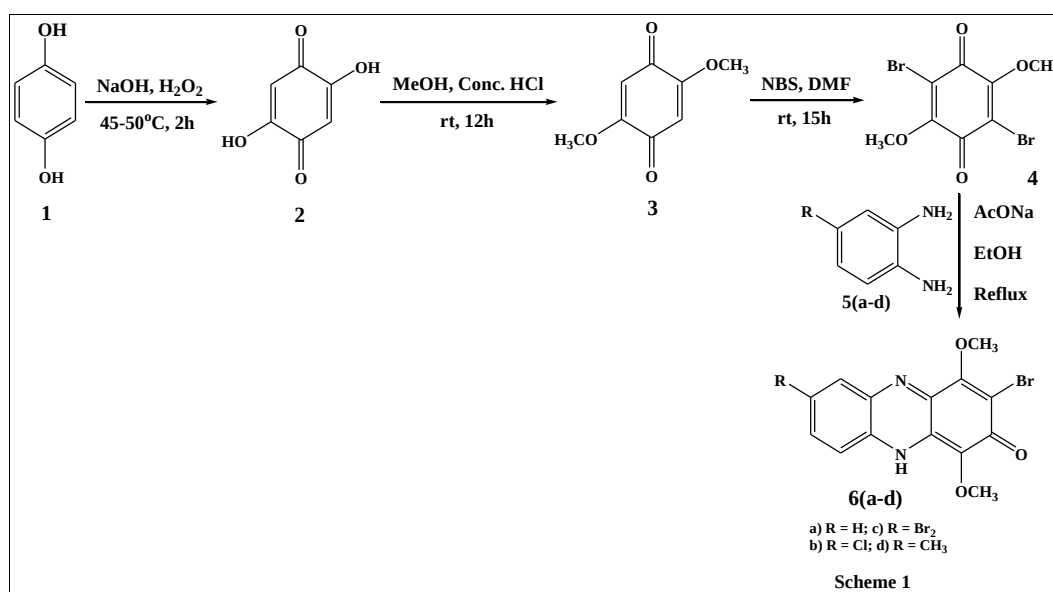


Fig 1: Synthetic Route for the Preparation of Benzo[a]phenazine Derivatives (6a-d)

General procedure for the synthesis of compounds (6a-d)

A solution of the appropriate substituted *ortho*-phenylenediamine (5a-d, 6.135 mmol) in anhydrous ethanol (30 mL) was charged into a 100 mL round-bottom flask. Anhydrous sodium acetate (6.135 mmol) was added, and the resulting mixture was stirred at room temperature for 15 min. Subsequently, 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone (4, 6.135 mmol) was added portion-wise, and the reaction mixture was heated at reflux for 3 h while monitoring progress by thin-layer chromatography. Upon completion, the mixture was cooled to room temperature, poured into water, and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford a crude brown solid. Purification by silica gel column chromatography

using a gradient of ethyl acetate and petroleum ether yielded the pure target compounds (6a-d).

Synthesis of 3-bromo-1,4-dimethoxyphenazin-2(10H)-one (6a)

The compound 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone 4 (2.0 g, 6.135 mmol) and *ortho*-phenylenediamine 5a (0.67 g, 6.135 mmol) in presence of anhydrous AcONa (0.504 g, 6.135 mmol) were made to react as described in the general procedure to afford 6a. IR (KBr) ν_{max} (cm $^{-1}$): 3378, 3006, 2924, 2853, 1646, 1609, 1561, 1414, 1313, 1228, 1198, 1057, 770, 703; ^1H NMR (400 MHz, DMSO- d_6): δ 6.82-7.22 (4H, m, Ar-H), 3.88 (3H, s, OCH $_3$), 3.76 (3H, s, OCH $_3$), 3.58 (1H, br.s, NH); ^{13}C NMR (75 MHz, DMSO- d_6): δ 168.7, 155.3, 155.1,

136.6, 135.3, 134.2, 131.4, 128.1, 124.4, 116.9, 114.7, 111.6, 60.8, 60.1; ESI-MS m/z : 335 $[M+H]^+$; Anal. Calcd. for $C_{14}H_{11}BrN_2O_3$; C, 50.10; H, 3.35; N, 8.39. Found C, 50.14; H, 3.33; N, 8.41.

Synthesis of 3-bromo-7-chloro-1,4-dimethoxyphenazin-2(10H)-one (6b)

The representative compound was prepared by reacting 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **4** (2.0 g, 6.135 mmol) and 4-chloro-1,2-phenylenediamine **5b** (0.875 g, 6.135 mmol) in the presence of anhydrous AcONa (0.504 g, 6.135 mmol) as per the general procedure described and the same extended to get the compound **6(b)**. IR (KBr) ν_{max} (cm⁻¹): 3380, 3012, 2940, 2872, 1650, 1613, 1601, 1578, 1440, 1388, 1236, 1130, 1063, 798, 708; ¹H NMR (400 MHz, DMSO-*d*⁶): δ 7.02-7.38 (3H, m, Ar-H), 3.90 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 3.62 (1H, br.s, NH); ¹³C NMR (75 MHz, DMSO-*d*⁶): δ 168.4, 155.0, 135.6, 134.7, 134.1, 129.0, 128.6, 125.1, 124.8, 122.3, 116.5, 115.8, 111.8, 60.9, 60.3; ESI-MS m/z : 369 $[M+H]^+$; Anal. Calcd for $C_{14}H_{10}BrClN_2O_3$; C, 45.44; H, 2.79; N, 7.65. Found C, 45.54; H, 2.71; N, 7.60.

Synthesis of 3, 7-dibromo-1, 4-dimethoxyphenazin-2(10H)-one (6c)

2,5-Dibromo-3,6-dimethoxy-1,4 Benzoquinone **4** (2.0 g, 6.135 mmol) and 4-bromo-1,2-phenylenediamine **5c** (0.1148 g, 6.135 mmol) in the presence of anhydrous AcONa (0.504 g, 6.135 mmol) were made to react to afford **6c**. IR (KBr) ν_{max} (cm⁻¹): 3379, 3008, 2935, 2860, 1648, 1618, 1600, 1570, 1438, 1379, 1339, 1232, 1125, 1061, 781, 705; ¹H NMR (400 MHz, DMSO-*d*⁶): δ 7.00-7.70 (3H, m, Ar-H), 3.90 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 3.61 (1H, br.s, NH); ¹³C NMR (75 MHz, DMSO-*d*⁶): δ 168.6, 155.2, 136.2, 135.6, 135.4, 134.0, 131.1, 130.2, 120.0, 116.8, 116.4, 111.5, 60.7, 60.2; ESI-MS m/z : 413 $[M+H]^+$; Anal. Calcd for $C_{14}H_{10}Br_2N_2O_3$; C, 40.58; H, 2.39; N, 6.82. Found C, 40.55; H, 2.45; N, 6.74.

Synthesis of 3-bromo-1, 4-dimethoxy-7-methylphenazin-2(10H)-one (6d)

The title compound was prepared from 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **4** (2.0 g, 6.135 mmol) 4-methyl-1,2-phenylenediamine **5d** (0.75 g, 6.135 mmol) and anhydrous AcONa (0.504g, 6.135 mmol) were made to react as described in the general procedure to afford **6d**. IR (KBr) ν_{max} (cm⁻¹) 3375, 3002, 2928, 2862, 1643, 1603, 1594, 1555, 1435, 1371, 1315, 1226, 1124, 1057, 780, 701; ¹H NMR (400 MHz, DMSO-*d*⁶): δ 7.08-7.35 (3H, m, Ar-H), 3.89 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 3.4 (1H, br.s, NH), 2.32 (3H, s, CH₃); ¹³C NMR (75 MHz, DMSO-*d*⁶): δ

168.9, 155.3, 135.5, 134.3, 133.6, 131.1, 128.8, 127.9, 125.0, 116.6, 114.7, 111.6, 60.6, 60.1, 21.3; ESI-MS m/z : 349 $[M+H]^+$; Anal. Calcd for $C_{15}H_{13}BrN_2O_3$; C, 51.09; H, 3.80; N, 7.95. Found C, 51.11; H, 3.74; N, 7.88.

Results and discussion

The synthesis of 7-substituted 3-bromo-1,4-dimethoxyphenazin-2(10H)-ones (**6a-d**) was achieved via a multi-step sequence from hydroquinone. Condensation of 2, 5-dibromo-3, 6-dimethoxy-1,4-benzoquinone with various *ortho*-phenylenediamines **5(a-d)** afforded targets **6a-d** in 66%-88% yields. The structural variations influenced physical profiles, with the methyl derivative **6d** giving the highest yield (88%, m.p. 208-210 °C). Full regioselective condensation was confirmed by comprehensive spectral data. The IR spectra displayed characteristic carbonyl stretching vibrations (C=O) at 1650-1640 cm⁻¹ and prominent C=N bands near 1620-1600 cm⁻¹. In ¹H NMR, the distinct singlet integrating for six protons at δ 3.80-3.90 ppm established the integrity of the two intact methoxy groups (OCH₃). Furthermore, ¹³C NMR spectra verified the core architecture, revealing the characteristic carbonyl carbon (C=O) signal at δ 169-168 ppm, C-OCH₃ carbon signal at δ 155-155.3 ppm, C-Br carbon signal at δ 120-114 ppm and aliphatic-OCH₃ carbon signal at δ 61-60 ppm. Mass spectrometry (ESI-MS) confirmed all molecular weights, displaying characteristic isotopic patterns including distinctive 1:1 doublets for monobrominated derivatives and 1:2:1 triplets for dibrominated analogs which definitively verified the specific bromine atom count in each synthesized molecule.

Conclusion

In conclusion, a straightforward and highly practical protocol for the synthesis of novel 7-substituted 3-bromo-1, 4-dimethoxyphenazin-2(10H)-one derivatives was successfully established starting directly from commercial hydroquinone. The multi-step sequence successfully utilized optimized reaction conditions, including mild regioselective bromination via N-bromosuccinimide and sodium acetate-assisted reflux condensation, producing the targeted frameworks in moderate to excellent yields (66%-88%). Detailed analytical and spectroscopic data fully confirmed the high purity and explicit structural identity of all novel derivatives. Given the well-established biomedical relevance of functionalized tricyclic heterocycles, these newly developed bromine- and methoxy-functionalized phenazinone scaffolds represent robust, highly promising chemical candidates for future deep biological screening against complex microbial pathogens.

Table 1: Physical Properties of Synthesized Phenazinone Derivatives

Compound Code	Chemical Name	Substituent (R)	Physical Appearance	Melting Point (°C)	Yield (%)
6a	3-bromo-1,4-dimethoxyphenazin-2(10H)-one	-H	Thick blue solid	257-259	75%
6b	3-bromo-7-chloro-1,4-dimethoxy phenazin-2(10H)-one	-Cl	Thick brown solid	225-227	70%
6c	3,7-dibromo-1,4-dimethoxyphenazin-2(10H)-one	-Br	Brown solid	278-280	66%
6d	3-bromo-1,4-dimethoxy-7-methylphenazin-2(10H)-one	-CH ₃	Brown solid	208-210	88%

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