Scientific paper

Synthesis and Antibacterial Activity of Vanillic Acid Derivatives Containing Indole Structure

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Abstract

Using indole-2-carboxylic acid and vanillic acid as raw materials, a series of 11 novel compounds (**7a**–**k**) were synthesized by chemically linking indole-2-carboxylic acid and vanillic acid through an aminothioureido group. The antibacterial activity of these compounds against *Vibrio harveyi* and *Vibrio parahaemolyticus* was evaluated using the agar perforating method. The results indicated that compounds **7g**–**j** exhibited superior antibacterial activity against both strains compared to the positive control, with **7g** showing the best performance. Structure-activity relationship analysis suggested that alkylation of the nitrogen atom in the indole ring could enhance the antibacterial effect, and shorter carbon chains were associated with better antibacterial activity.

Keywords: Vanillic acid; Indole; *Vibrio harveyi*; *Vibrio parahaemolyticus*

1. Introduction

Vibrio species are Gram-negative bacteria and represent one of the most common bacterial groups in marine environments.[1] They are also among the major pathogens affecting aquaculture species. To date, more than 100 species of *Vibrio* have been identified globally, with *Vibrio harveyi* and *Vibrio parahaemolyticus* being two of the most prevalent pathogenic strains. These bacteria not only cause diseases and mortality in farmed aquatic animals such as fish, shrimp, and crabs, leading to significant economic losses in aquaculture, but they can also infect humans and other animals.[2] Consumption of aquaculture products contaminated with pathogenic *Vibrio* species can pose a health risk to humans.[3–5] The use of antibiotics is currently the primary treatment for *Vibrio* infections in aquaculture. However, the extensive use of antibiotics has led to significant antibiotic residues in the environment and contributes to the development of bacterial resistance.[6]

Vanillic acid (VA), chemically known as 4-hydroxy-3-methoxybenzoic acid with a molecular weight of 168.15 g/mol, is a white to pale yellow solid powder. VA is a phenolic acid derivative naturally found in several medicinal plants, including dandelion, *Angelica sinensis*, and olive.[7] As a natural bioactive compound, vanillic acid exhibits a high degree of safety and is often used as a food additive to enhance flavor due to its distinctive aroma.[8] In addition, vanillic acid and its derivatives possess various biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties.[9–12] Indole, also known as benzopyrrole, is a typical benzene-fused heterocyclic compound, and numerous studies have shown that the indole structure and its derivatives exhibit strong antimicrobial activity.[13–14]

Based on the above analysis, this study utilizes indole-2-carboxylic acid and vanillic acid as starting materials and employs acylthiourea to conjugate the indole structure with vanillic acid in a single molecular framework (Figure 1). The study also investigates the impact of substituents on the indole benzene ring or the substitution of hydrogen at the nitrogen atom on the antimicrobial activity of the resulting compounds. The aim is to identify compounds with potent antimicrobial properties, providing an experimental foundation for the development of novel antibacterial agents.

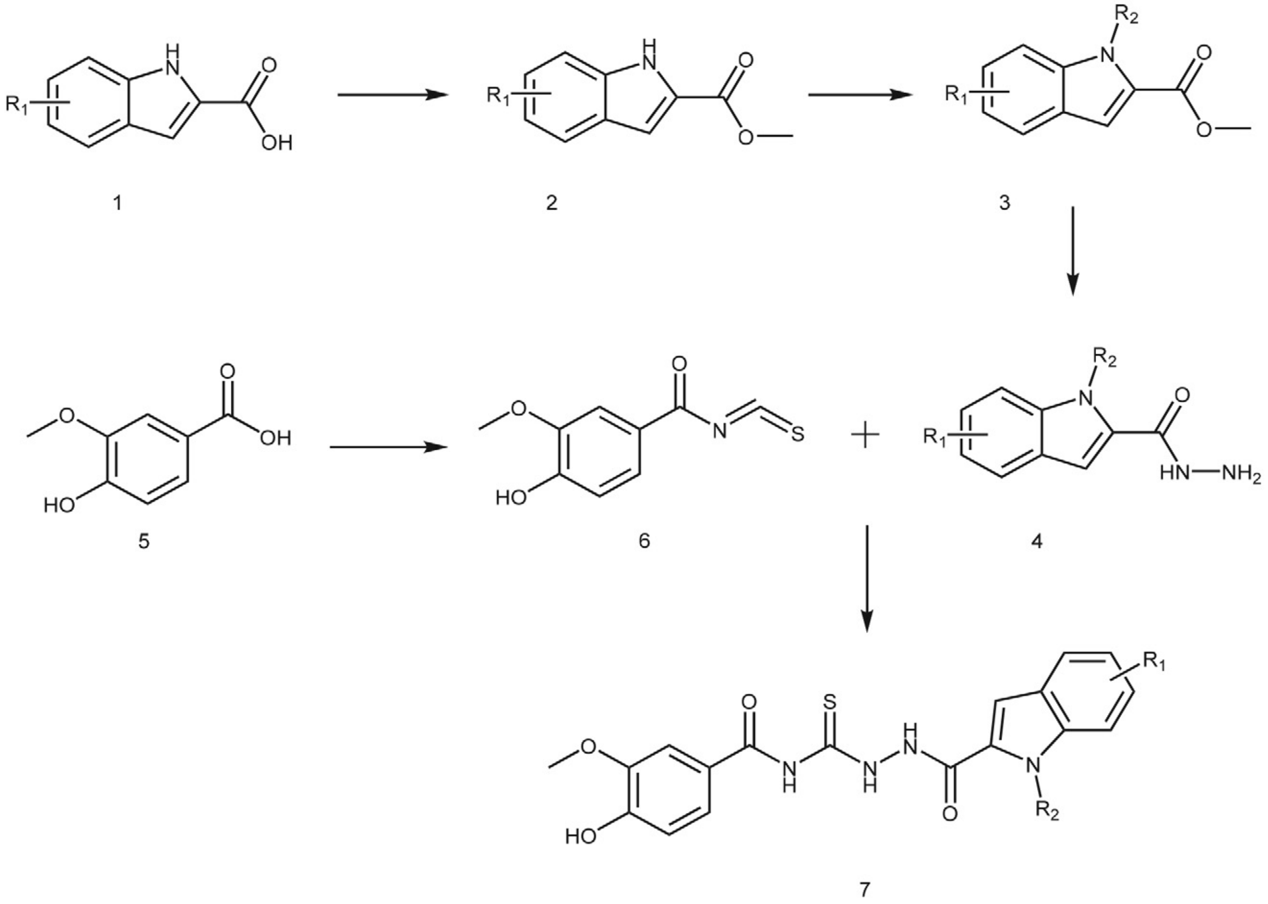

Figure 1. Synthetic route of vanillic acid derivatives containing indole structure

2. Experimental Methods

2. 1. Experimental Instruments

The melting points were determined in opened capillaries on the Yanaco melting point apparatus of model X-4 and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Cary 630 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE NEO 400 MHz spectrometer using DMSO-d_6 as the solvent at room temperature. The compounds were analyzed using high-resolution mass spectrometry on a Waters G2-S QTof. *Vibrios* were purchased from the China General Microbial Strain Preservation and Management Center. Synthetic reagents and all chemical substances were bought from commercial sources and were used without any purification unless otherwise stated.

2. 2. Synthesis of Compounds 2–7

Preparation of methyl ester of indole-2-carboxylic acid substituted on the benzene ring 2. The compound substituted indole-2-carboxylic acid **1** (3 mmol) was weighed and placed in a 50 mL round-bottom flask. To this, 20 mL of methanol was added to dissolve the solid. Then, 50 μL of concentrated sulfuric acid was introduced, and the reaction mixture was refluxed at 70 °C. The progress of the reaction was monitored by TLC until completion. Upon reaction completion, the solvent was removed under reduced pressure, and the residue was dissolved in water. The pH was adjusted to 8–9, and the resulting mixture was extracted with ethyl acetate. The organic phase was then evaporated under reduced pressure to obtain the pure product **2**.

Preparation of benzene ring-substituted-*N*-substituted-indole-2-carboxylic acid methyl ester 3. Weigh 2.8 mmol of the corresponding amount of compound **2** and dissolve it in 10 mL of anhydrous ethanol. At room temperature, add 0.108 g (4.2 mmol) of sodium hydride (NaH) to the solution and stir for 15 minutes. Then, add 2.8 mmol of the alkyl bromide to the reaction mixture. The reaction progress was monitored by TLC until completion. After the reaction, add an appropriate amount of water to the mixture, extract with ethyl acetate, and concentrate under reduced pressure. The product **3** was purified by column chromatography (ethyl acetate : petroleum ether = 1:30) to obtain the pure compound.

Preparation of benzene ring-substituted indole-2-acylhydrazine 4. Weigh 2.5 mmol of the corresponding amount of compound **3**, and heat to dissolve it in 20 mL of anhydrous ethanol. To the reaction mixture, add 2 mL of 80% hydrazine hydrate ($NH_2NH_2{\cdot}H_2O$), and reflux at 78 °C. The reaction progress was monitored by TLC until completion. Afterward, cool the reaction mixture in an ice bath to precipitate the solid product **4**.

Preparation of vanillic acid isothiocyanate (6). In a 100 mL round-bottom flask, 40 mL of ethyl acetate was added, and the mixture was cooled in an ice bath. To this, triphenylphosphine (TPP) (1.94 g, 7.4 mmol), trichloroisocyanuric acid (TCCA) (0.512 g, 2.2 mmol), and vanillic acid (**5**) (1 g, 6 mmol) were sequentially added and stirred for 15 minutes. Potassium thiocyanate (KSCN) (1.44 g, 15 mmol) was then introduced, and the mixture was stirred for several minutes before removing the ice bath. The reaction was allowed to proceed overnight at room temperature. The product **6** was purified by column chromatography using a mixture of ethyl acetate and petroleum ether (1:4) as the eluent.

Preparation of *N*-(2-(phenyl substituted-1-substituted-indole-2-carbonyl)hydrazine-1-isothiocyanate)-4-hydroxy-3-methoxybenzamide 7. In a 50 mL round-bottom flask, 10 mL of 1,4-dioxane was added, followed by the addition of vanillic acid isothiocyanate (**6**) (0.2 g, 0.96 mmol) and an equimolar amount of compound **4**. The reaction was conducted at 60 °C and monitored by TLC until completion. After cooling the reaction mixture, petroleum ether was added to induce precipitation of the solid product. The solid was filtered, dried, and recrystallized from a mixture of ethyl acetate and petroleum ether to obtain the pure product **7** (Table 1).

Table 1. The yields of the target compounds **7**

Compound	R_1	R_2	Yield (%)	Product Weight (g)
7a	–	–H	86	0.317
7b	5-F	–H	74	0.286
7c	5-Br	–H	71	0.315
7d	5-OCH_3	–H	78	0.310
7e	6-Br	–H	75	0.333
7f	–	Bn	74	0.337
7g	–	Et	85	0.336
7h	–	Pr	83	0.339
7i	–	i-Bu	79	0.334
7j	–	n-Bu	78	0.329
7k	–	n-Pe	75	0.327

***N*-(2-(1*H*-Indole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7a**). Pale yellow solid, yield 86%, m.p. 232–233 °C. IR (KBr) *ν* 3365, 3299, 3052, 1669, 1604, 1577, 1513, 1454, 1194, 1139, 1030, 740 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) *δ* 12.48 (s, 1H, NH), 11.80 (s, 1H, NH), 11.55 (s, 1H, NH), 11.09 (s, 1H, NH), 10.08 (s, 1H, OH), 7.71–7.64 (m, 2H, Ar-H), 7.60 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.33 (s, 1H, H in indole), 7.23 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.08 (t, *J* = 7.5 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.3 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 181.69, 167.52, 159.76, 152.24, 147.63, 137.24, 129.44, 127.39, 124.40, 123.95, 122.47, 122.31, 120.49, 115.64, 112.88, 104.73, 56.29. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{16}N_4O_4SNa^+$: 407.0790. Found: 407.0792.

***N*-(2-(5-Fluoro-1*H*-indole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7b**). White solid, yield 74%, m.p. 242–244 °C. IR (KBr) *ν* 3396, 3314, 3083, 1673, 1606, 1574, 1517, 1454, 1240, 1210, 1168, 1029 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) *δ* 12.46 (s, 1H, NH), 11.91 (s, 1H, NH), 11.55 (s, 1H, NH), 11.15 (s, 1H, NH), 10.08 (s, 1H, OH), 7.66 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar-H), 7.54–7.42 (m, 2H, Ar-H), 7.31 (d, *J* = 2.3 Hz, 1H, H in indole), 7.10 (td, *J* = 9.2, 2.5 Hz, 1H, Ar-H), 6.91 (d, *J* =8.3 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 181.84, 167.49, 159.49, 158.87, 156.55, 152.24, 147.62, 133.98, 131.16, 127.52, 127.41, 123.95, 122.46, 115.64, 114.15, 114.06, 113.33, 113.06, 112.89, 106.55, 106.32, 104.69, 104.63, 56.28. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{15}FN_4O_4SNa^+$: 425.0696. Found: 425.0696.

***N*-(2-(5-Bromo-1*H*-indole-2-carbonyl)hydrazine-1- carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7c**). Pale yellow solid. yield 71%, m.p. 263–264 °C. IR (KBr) *ν* 3378, 3311, 3102, 1669, 1603, 1578, 1509, 1450, 1206, 1150, 1034, 529 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) *δ* 12.45 (s, 1H, NH), 12.02 (s, 1H, NH), 11.56 (s, 1H, NH), 11.20 (s, 1H, NH), 10.08 (s, 1H, OH), 7.93 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.66 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.4, 2.2 Hz, 1H, Ar-H), 7.43 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.38–7.33 (m, 1H, Ar-H), 7.32 (s, 1H, H in indole), 6.91 (d, *J* = 8.4 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 181.83, 167.50, 159.42, 152.24, 147.62, 135.82, 130.78, 129.18, 126.98, 124.49, 123.95, 122.45, 115.64, 114.90, 112.92, 112.89, 104.12, 56.28. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{15}BrN_4O_4SNa^+$. 484.9895; Found: 484.9890.

***N*-(2-(5-Methoxy-1*H*-indole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7d**). White solid, yield 78%, m.p. 241–244 °C. IR (KBr) *ν* 3428, 3310, 3004, 1653, 1608, 1585, 1497, 1450, 1210, 1158, 1033, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) *δ* 12.47 (s, 1H, NH), 11.65 (s, 1H, NH), 11.54 (s, 1H, NH), 11.04 (s, 1H, NH), 10.08 (s, 1H, OH), 7.66 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.4, 2.3 Hz, 1H, Ar-H), 7.35 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.24 (s, H, H in indole), 7.14 (d, *J* = 2.7 Hz, 1H, Ar-H), 6.94–6.85 (m, 2H, Ar-H), 3.89 (s, 3H, OCH_3), 3.78

(s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.61, 167.52, 159.69, 154.37, 152.23, 147.63, 132.57, 129.68, 127.71, 123.94, 122.46, 115.78, 115.63, 113.71, 112.88, 104.45, 102.53, 56.28, 55.73. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{19}H_{18}FN_4O_5SNa^+$: 437.0896. Found: 437.0889.

***N*-(2-(6-Bromo-1*H*-indole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7e**). White solid, yield 75%, m.p. 244–245 °C. IR (KBr) ν 3415, 3267, 3006, 1670, 1604, 1503, 1452, 1196, 1120, 1031, 591 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H, NH), 11.95 (s, 1H, NH), 11.55 (s, 1H, NH), 11.18 (s, 1H, NH), 10.08 (s, 1H, OH), 7.70–7.62 (m, 3H, Ar-H), 7.60 (dd, J = 8.3, 2.2 Hz, 1H, Ar-H), 7.35 (s, 1H, H in indole), 7.21 (dd, J = 8.6, 1.8 Hz, 1H, Ar-H), 6.90 (d, J = 8.3 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.88, 167.48, 159.45, 152.24, 147.62, 137.92, 130.37, 126.41, 124.26, 123.95, 123.54, 122.45, 117.18, 115.63, 115.27, 112.88, 104.77, 56.28. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{15}BrN_4O_4SNa^+$: 484.9895. Found: 484.9897.

***N*-(2-(1-Benzylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7f**). Pale yellow solid, yield 74%, m.p. 138–140 °C. IR (KBr) ν 3389, 3184, 3029, 1670, 1605, 1513, 1451, 1280, 1191, 1029, 742 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H, NH), 11.56 (s, 1H, NH), 11.17 (s, 1H, NH), 10.07 (s, 1H, OH), 7.73 (d, J = 7.8 Hz, 1H, Ar-H), 7.65 (d, J = 2.2 Hz, 1H, Ar-H), 7.62–7.52 (m, 2H, Ar-H), 7.40 (s, 1H, H in indole), 7.31–7.07 (m, 5H, Ar-H), 6.90 (d, J = 8.4 Hz, 1H, Ar-H), 5.85 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.95, 167.52, 160.50, 152.24, 147.62, 138.90, 138.75, 129.43, 128.83, 127.52, 127.31, 126.28, 124.86, 123.93, 122.57, 122.47, 121.12, 115.64, 112.88, 111.66, 107.40, 56.28, 47.43. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{22}N_4O_4SNa^+$: 497.1259. Found: 497.1262.

***N*-(2-(1-Ethylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7g**). White solid, yield 85%, m.p. 149–152 °C. IR (KBr) ν 3378, 3295, 2971, 1670, 1601, 1508, 1450, 1283, 1196, 1029, 742 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H, NH), 11.57 (s, 1H, NH), 11.06 (s, 1H, NH), 10.08 (s, 1H, OH), 7.71 (d, J = 7.9 Hz, 1H, Ar-H), 7.66 (d, J = 2.2 Hz, 1H, Ar-H), 7.60 (dd, J = 8.4, 2.2 Hz, 2H, Ar-H), 7.31 (t, J = 7.6 Hz, 1H, Ar-H), 7.28 (s, 1H, H in indole), 7.13 (t, J = 7.5 Hz, 1H, Ar-H), 6.91 (d, J = 8.3 Hz, 1H, Ar-H), 4.57 (q, J = 7.0 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 1.30 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 182.01, 167.56, 160.37, 152.24, 147.62, 138.06, 129.17, 126.16, 124.59, 123.94, 122.51, 122.48, 120.80, 115.65, 112.88, 110.96, 106.64, 56.29, 39.40, 16.20. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{20}H_{20}N_4O_4SNa^+$: 435.1103. Found: 435.1104.

***N*-(2-(1-Propylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7h**). Pale yellow solid, yield 83%, m.p. 121–123 °C. IR (KBr) ν 3390, 3193, 2962, 1670, 1606, 1513, 1460, 1289, 1192, 1031, 742 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H, NH), 11.56 (s, 1H, NH), 11.06 (s, 1H, NH), 10.08 (s, 1H, OH), 7.70 (d, J = 7.9 Hz, 1H, Ar-H), 7.66 (d, J = 2.2 Hz, 1H, Ar-H), 7.60 (dd, J = 8.4, 2.3 Hz, 2H, Ar-H), 7.31 (t, J = 8.2 Hz, 1H, Ar-H), 7.28 (s, 1H, H in indole), 7.13 (t, J = 7.5 Hz, 1H, Ar-H), 6.91 (d, J = 8.4 Hz, 1H, Ar-H), 4.50 (t, J = 7.3 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 1.73 (h, J = 7.4 Hz, 2H, CH_2), 0.83 (t, J = 7.4 Hz, 3H, CH_3). 13C NMR (101 MHz, DMSO-d_6) δ 181.96, 167.56, 160.44, 152.24, 147.62, 138.63, 129.48, 126.01, 124.52, 123.93, 122.49, 122.44, 120.76, 115.64, 112.88, 111.26, 106.69, 56.28, 45.76, 24.00, 11.58. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{21}H_{22}N_4O_4SNa^+$: 449.1259. Found: 449.1261.

***N*-(2-(1-Isobutylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7i**). Pale yellow solid, yield 79%, m.p. 126–128 °C. IR (KBr) ν 3390, 3192, 2959, 1669, 1606, 1514, 1458, 1274, 1190, 1032, 741 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H, NH), 11.56 (s, 1H, NH), 11.07 (s, 1H, NH), 10.08 (s, 1H, OH), 7.70 (d, J = 7.9 Hz, 1H, Ar-H), 7.66 (d, J = 2.2 Hz, 1H, Ar-H), 7.60 (dd, J = 8.4, 2.3 Hz, 2H, Ar-H), 7.29 (d, J = 16.6 Hz, 1H, Ar-H), 7.29 (s, 1H, H in indole), 7.12 (t, J = 7.5 Hz, 1H, Ar-H), 6.91 (d, J = 8.3 Hz, 1H, Ar-H), 4.39 (d, J = 7.5 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 2.22–2.04 (m, 1H, CH), 0.80 (d, J = 6.7 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.90, 167.57, 160.56, 152.24, 147.62, 139.03, 129.71, 125.91, 124.44, 123.93, 122.39, 120.71, 115.64, 112.88, 111.69, 106.95, 56.28, 51.04, 29.99, 20.32. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{24}N_4O_4SNa^+$: 463.1416. Found: 463.1419.

***N*-(2-(1-Butylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7j**). Pale yellow solid, yield 78%, m.p. 120–122 °C. IR (KBr) ν 3390, 3199, 2956, 1670, 1606, 1513, 1463, 1289, 1192, 1031, 742 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H, NH), 11.55 (s, 1H, NH), 11.05 (s, 1H, NH), 10.08 (s, 1H, OH), 7.70 (d, J = 7.8 Hz, 1H, Ar-H), 7.66 (d, J = 2.3 Hz, 1H, Ar-H), 7.59 (td, J = 5.6, 2.8 Hz, 2H, Ar-H), 7.30 (t, J = 7.7 Hz, 1H, Ar-H), 7.27 (s, 1H, H in indole), 7.13 (t, J = 7.5 Hz, 1H, Ar-H), 6.91 (d, J = 8.3 Hz, 1H, Ar-H), 4.53 (t, J = 7.3 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 1.69 (p, J = 7.6 Hz, 2H, CH_2), 1.26 (h, J = 7.2 Hz, 2H, CH_2), 0.87 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 182.00, 167.56, 160.45, 152.24, 147.62, 138.55, 129.44, 126.03, 124.53, 123.93, 122.48, 122.45, 120.75, 115.64, 112.88, 111.19, 106.72, 56.28, 44.11, 32.89, 20.03, 14.19. HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{22}H_{24}N_4O_4SNa^+$: 463.1416. Found: 463.1415.

***N*-(2-(1-Pentylindole-2-carbonyl)hydrazine-1-carbonothioyl)-4-hydroxy-3-methoxybenzamide** (**7k**). Pale yellow solid, yield 75%, m.p. 130–132 °C. IR (KBr) ν 3390, 3195, 2955, 1670, 1606, 1513, 1464, 1289, 1193, 1031, 742 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H, NH), 11.55 (s, 1H, NH), 11.05 (s, 1H, NH), 10.08 (s, 1H, OH), 7.70 (d, J = 7.9 Hz, 1H, Ar-H), 7.66 (d, J = 2.2 Hz, 1H, Ar-H), 7.63–7.55 (m, 2H, Ar-H), 7.30 (t, J = 7.7 Hz, 1H, Ar-H), 7.27 (s, 1H, H in indole), 7.13 (t, J = 7.5 Hz, 1H, Ar-H), 6.91 (d, J = 8.3 Hz, 1H, Ar-H), 4.53 (t, J = 7.3 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 1.71 (p, J = 7.6 Hz, 2H, CH_2), 1.35–1.16 (m, 4H, CH_2), 0.83 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 182.04, 167.55, 160.46, 152.24, 147.62, 138.53, 129.44, 126.03, 124.53, 123.93, 122.48, 122.45, 120.75, 115.64, 112.88, 111.18, 106.70, 56.28, 44.28, 30.44, 28.90, 22.38, 14.38. HRMS (ESI) m/z [M+Na]$^+$ calcd for $C_{23}H_{26}N_4O_4SNa^+$: 477.1572. Found: 477.1568.

2. 3. Antibacterial Activity Assay

The antibacterial activity of compounds **7a–k** against *Vibrio harveyi* and *Vibrio parahaemolyticus* was evaluated using the agar perforating method.[15–16] Each compound was dissolved in DMSO to a concentration of 1 mg/mL, while streptomycin sulfate was prepared in water at the same concentration (1 mg/mL) as a positive control.

V. harveyi and *V. parahaemolyticus* were cultured in liquid medium for several hours and then prepared for inoculation. Solid MH agar plates were prepared by heating the medium to boiling, allowing it to cool slightly before pouring approximately 30 mL of the medium into each plate under sterile conditions. After the agar solidified at room temperature, the bacterial suspension was evenly spread across the surface of the solid medium using a sterile cotton swab and allowed to stand for 5 minutes. Wells were then punched into the solidified agar using a sterile puncher. A 50 µL volume of the 1 mg/mL sample solution was added to each well, with three replicate wells for each sample. The plates were incubated at 32 °C for 10–12 hours, and the diameter of the inhibition zones was measured to assess antibacterial activity.

3. Results and Discussion

The antibacterial activity of the compounds against *V. harveyi* and *V. parahaemolyticus* is summarized in Table 2. As shown, compounds **7g–k** exhibited minimum inhibitory concentrations (MIC) against *V. harveyi* equal to or greater than that of the positive control. Compounds **7g–j** also demonstrated comparable or superior inhibitory effects against *V. parahaemolyticus* relative to the positive control. Notably, compound **7g** exhibited the most potent antibacterial activity against both *V. harveyi* and *V. parahaemolyticus*, with MIC of 0.0039 mg/mL and 0.0313 mg/mL, respectively.

The results of the synthesized compounds suggest that the presence of substituents on the indole phenyl ring tends to reduce the inhibitory activity against *Vibrio* species. Furthermore, the solubility of the compounds containing substituents was found to be lower compared to those without substituents. Modification of the nitrogen atom in the indole ring through alkylation with aliphatic chains was found to enhance antibacterial activity, with shorter carbon chains resulting in better activity. Among compounds with the same number of carbon atoms (**7i**, **7j**), a shorter alkyl chain at the nitrogen atom led to a more pronounced inhibitory effect against *Vibrio* species.

4. Conclusion

In this study, we report the synthesis of eleven novel vanillic acid derivatives **7** containing an indole structure. The structures of all compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMMR and HR-MS (ESI). Among the synthe-

Table 2. *In vitro* antibacterial activity of the target compounds against *Vibrio harveyi* and *Vibrio parahaemolyticus*

Compound	**Anti-*Vibrio harveyi* activity**		**Anti-*Vibrio parahaemolyticus* activity**	
	Diameter^a (mm)	**MIC (mg/mL)**	**Diameter^a(mm)**	**MIC (mg/mL)**
7a	18.72±0.19	0.0313	15.27±0.31	0.25
7b	18.43±0.12	0.0313	14.70±0.26	–
7c	14.32±0.24	–	12.80±0.32	–
7d	15.40±0.09	–	11.86±0.17	–
7e	16.86±0.18	0.0625	14.15±0.28	–
7f	16.45±0.11	0.125	13.30±0.25	0.25
7g	21.54±0.16	0.0039	16.90±0.16	0.0313
7h	19.77±0.23	0.0039	16.13±0.28	0.0313
7i	19.25±0.25	0.0039	16.04±0.19	0.0313
7j	16.59±0.34	0.0078	14.51±0.28	0.0625
7k	15.80±0.12	0.0156	13.32±0.17	0.125
DMSO	6.00	–	6.00	–
H_2O	6.00	–	6.00	–
Streptomycin sulfate	–	0.0156	–	0.0625

sized compounds, compound **7g** exhibited the strongest inhibitory activity against *Vibrio harveyi* and *Vibrio parahaemolyticus*, surpassing the inhibitory effect of the positive control drug. Additionally, we discuss the relationship between the structure of the compounds and their inhibitory effects on *Vibrio* species, and find that modification of the nitrogen in the indole ring enhances the antimicrobial activity. This provides an experimental basis for the development of new antimicrobial agents in future research.

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References

1. L. Girard, *Crit. Rev. Microbiol.* **2019**, *45*, 451–471. **DOI:**10.1080/1040841X.2019.1624499
2. B. Austin, *Vet. Microbiol.* **2010**, *140*, 310–317. **DOI:**10.1016/j.vetmic.2009.03.015
3. A. Sampaio, V. Silva, P. Poeta, F. Aonofriesei, *Diversity* **2022**, *14*, 97. **DOI:**10.3390/d14020097
4. V. Letchumanan, K. G. Chan, L. H. Lee, *Front. Microbiol.* **2014**, *5*, 705. **DOI:**10.3389/fmicb.2014.00705
5. X. H. Zhang, X. He, B. Austin, *Mar. Life Sci. Tech.* **2020**, *2*, 231–245. **DOI:**10.1007/s42995-020-00037-z
6. A. M. Helmi, A. T. Mukti, A. Soegianto, M. H. Effendi, *Sys. Rev. Pharm.* **2020**, *11*, 51–58. **DOI:** 10.31838/srp.2020.8.8
7. A. S. Ingole, M. P. Kadam, A. P. Dalu, S. M. Kute, P. R. Mange, V. D. Theng, O. R. Lahane, A. P. Nikas, Y. V. Kawal, S. U. Nagrik, P. A. Patil, *J. Drug Deliv. Ther.* **2021**, *11*, 200–204. **DOI:**10.22270/jddt.v11i2-S.4823
8. J. Kaur, M. Gulati, S. K. Singh, G. Kuppusamy, B. Kapoor, V. Mishra, S. Gupta, M. F. Arshad, O. Porwal, N. K. Jha, M. V. N. L. Chaitanya, D. K. Chellappan, G. Gupta, P. K. Gupta, K. Dua, R. Khursheed, A. Awasthi, L. Corrie, *Trends Food Sci. Tech.* **2022**, *122*, 187–200. **DOI:**10.1016/j.tifs.2022.02.023
9. I. M. Oke, L. M. Ramorobi, S. S. Mashele, S. L. Bonnet, T. J. Makhafola, K. C. Eze, A. E. M. Noreljaleel, C. I. Chukwuma, *J. Pharm. Pharmacol.* **2021**, *73*, 1703–1714. **DOI:**10.1093/jpp/rgab086
10. H. A. Adekola, A. O. Adeleye, T. O. Adesetan, J. B. Folorunso, F. Odeyemi, *Microbes Infect. Chemother.* **2022**, *2*, 1548–1548. **DOI:**10.54034/mic.e1548
11. C. Calixto-Campos, T. T. Carvalho, M. S. N. Hohmann, M. S. N. Hohmann, F. A. Pinho-Ribeiro, V. Fattori, M. F. Manchope, A. C. Zarpelon, M. M. Baracat, S. R. Georgetti, R. Casagrande, W. A. Verri, *J. Nat. Prod.* **2015**, *78*, 1799–1808. **DOI:**10.1021/acs.jnatprod.5b00246
12. B. Singh, A. Kumar, H. Singh, S. Kaur, S. Arora, B. Singh, *Phytother. Res.* **2022**, *36*, 1338–1352. **DOI:**10.1002/ptr.7392
13. H. Li, S. Wu, X. Yang, H. He, Z. Wu, B. Song, R. Song, *J. Agr. Food. Chem.* **2022**, *70*, 12341–12354. **DOI:**10.1021/acs.jafc.2c04213
14. W. Hong, J. Li, Z. Chang, X. Tan, H. Yang, Y. Ouyang, Y. Yang, S. Kaur, I. C. Paterson, Y. F. Ngeow, H. Wang, *J. Antibiot.* **2017**, *70*, 832–844. **DOI:**10.1038/ja.2017.55
15. X. Wang, J. Du, J. Zhou, *Ind. Crop. Prod.* **2019**, *133*, 409–413. **DOI:**10.1016/j.indcrop.2019.02.050
16. Y. Hao, J. Huo, T. Wang, G. Sun, W. Wang, *J. Pharmaceut. Biomed. Anal.* **2020**, *180*, 113089. **DOI:**10.1016/j.jpba.2019.113089

Povzetek

Z uporabo indol-2-karboksilne kisline in vanilinske kisline kot izhodnih spojin smo pripravilo serijo 11 novih spojin (**7a**–**k**). Sinteze so potekale tako, da smo povezali indol-2-karboksilni fragment in vanilinski fragment s pomočjo aminotioureidne skupine. S pomočjo metode na agarju smo določili antibakterijsko delovanje teh spojin proti *Vibrio harveyi* and *Vibrio parahaemolyticus*. Rezultati kažejo, da spojine **7g**–**j** izkazujejo aktivnosti proti obema organizmoma, ki so bile celo večje od aktivnosti pozitivne kontrolne spojine; spojina **7g** se je izkazala kot najbolj učinkovita. Primerjava strukture in aktivnosti kaže, da alkiliranje dušikovega atoma v indolnem obroču lahko poveča antibakterijski učinek; analogen učinek ima tudi krajšanje ogljikove verige.