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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF A BROMINATED SCHIFF BASE DERIVED FROM O-VANILLIN

Ruslan Guliyev
Baku State University, Baku, Azerbaijan

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This work presents the synthesis and characterization of 5-bromo-2-hydroxy-3-methoxybenzaldehyde and a new Schiff base obtained by condensation of this aldehyde and 4-methoxyaniline. The structures of both the aldehyde and the synthesized Schiff base were confirmed by ^{1}H and ^{13}C NMR spectral analyses. The biological activity of the Schiff base was evaluated against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa strains. Comparative antibacterial studies with ampicillin as a standard showed that the synthesized compound exhibits pronounced inhibitory activity against Escherichia coli and moderate activity against Pseudomonas aeruginosa and Staphylococcus aureus. This, in turn, indicates promising potential for further pharmaceutical research.

Keywords: Schiff base, biological activity, NMR spectroscopy, o-vanillin

INTRODUCTION

In the 19th century, the German chemist Hugo Schiff, while studying the reaction that forms aniline red, discovered crystalline byproducts. Analysis of these products led him to the discovery of a new class of organic compounds, which were later named Schiff bases. Schiff was the first to systematically study the interaction of primary amines with aliphatic and aromatic aldehydes and also established differences in their interactions with ketones [1-3].

Schiff bases are formed by a condensation reaction between carbonyl compounds and primary amines. The reaction typically occurs at room or elevated temperatures in the presence of acid or base catalysts. These compounds, known as azomethines or imines, exhibit a wide range of structural variations, which directly influences their physicochemical behavior and high reactivity [4-6]. The lone electron pair of the nitrogen atom in the imine group plays a special role, determining both the chemical activity and biological properties of these compounds. Schiff bases are widely used as precursors in organic synthesis to produce pharmaceutically and industrially significant compounds through reduction, substitution, cycloaddition, and intramolecular cyclization reactions [7-9].

The presence of a conjugated aromatic ring system and a C=N double bond in Schiff bases explains their intense color and pronounced optical properties. This makes them promising materials for use as organic dyes, luminescent compounds, fluorescent labels, and components of OLED displays [10-13].

E-mail address: ruslan.quliyev622@gmail.com

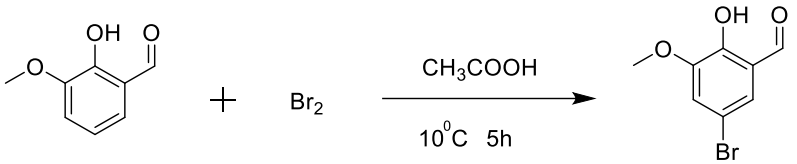
Numerous studies have demonstrated the high biological activity of Schiff bases and their derivatives. They exhibit a wide range of pharmacological effects, including antibacterial, antiviral, antifungal, antitumor, antihistamine, antituberculosis, antimalarial, antioxidant, and antipyretic properties [14-19].

In coordination chemistry, Schiff bases are considered ideal ligands for the formation of metal complexes. Of particular interest are compounds containing hydroxyl groups in the ortho position, which facilitate chelation and increase the stability of the complexes [20-23]. Metal complexes of Schiff bases have found wide application in biomimetic research, sensor systems, catalytic processes, and organic synthesis. In some cases, they exhibit greater biological activity than the parent ligands. This, in turn, is due to the involvement of the metal in electron transfer and stabilization of the active forms of the compounds [24-27].

Taking into account the above advantages of azomethine compounds, a new Schiff base based on ortho-vanillin derivative was synthesized and its antimicrobial activity was studied.

EXPERIMENTAL

In the first step, a bromination reaction was carried out to obtain a primary aldehyde (Scheme 1).



Scheme 1. Synthesis of 5-bromo-2-hydroxy-3-methoxy benzaldehyde

0,025 mol of o-vanillin is placed in a three-necked flask equipped with a reflux condenser, magnetic stirrer, magnet, and thermometer, and 30 ml of acetic acid is added. Then, at 10°C, 0,03 mol of bromine is added dropwise to the solution. After 5 hours, the reaction is poured onto ice, and a precipitate form. The precipitate is filtered, washed with water, and dried. Yield 82%.

Recrystallization in ethanol and acetic acid yields light yellow crystals. Yield 63%. Melting point 125-127°C.

^{1}H NMR spectrum (Figure 1): ($CDCl_3$, δ, ppm), 3.92 s (3H, OCH_3), 7.16-7.17 d (1H, $C_{Ar}H$), 7.30-7.31 d (1H, $C_{Ar}H$), 9.85 s (1H, CHO), 11.00 s (1H, OH).

^{13}C NMR spectrum (Figure 2): ($CDCl_3$, δ, ppm), 56.54 (OCH_3), 111.05 (C_{Ar}), 120.72 ($C_{Ar}H$), 121.30 (C_{Ar}), 126.11 ($C_{Ar}H$), 149.27 (C_{Ar}), 150.88 (C_{Ar}), 195.41 (CHO).

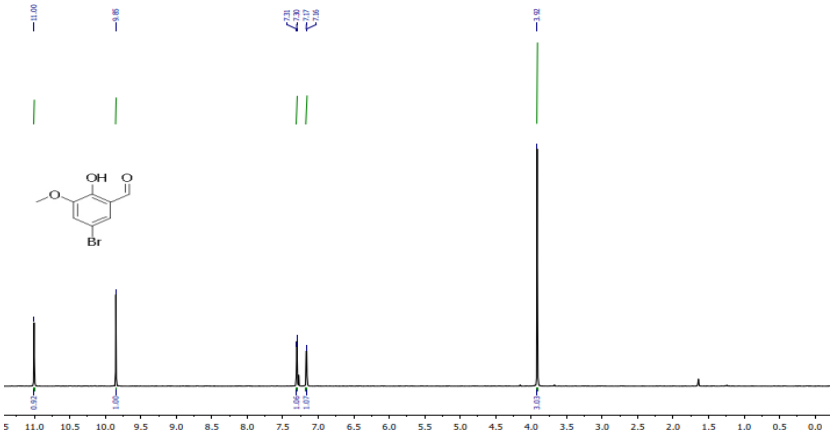


Figure 1. ^{1}H NMR spectrum of 5-bromo-2-hydroxy-3-methoxy benzaldehyde

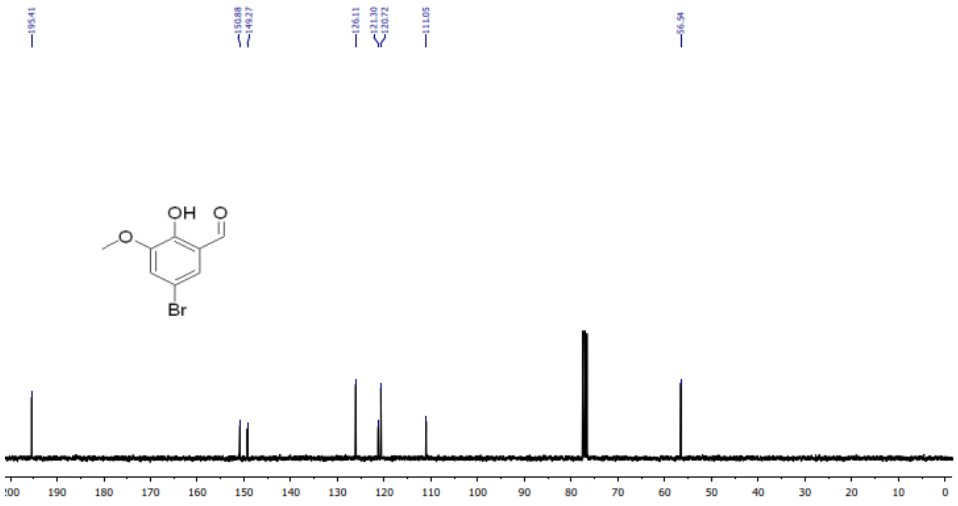
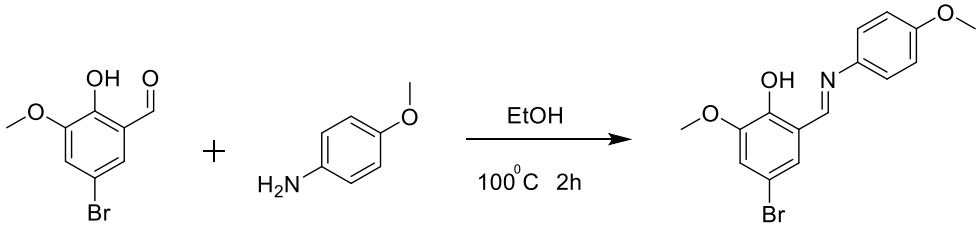


Figure 2. ^{13}C NMR spectrum of 5-bromo-2-hydroxy-3-methoxy benzaldehyde

The well-known method of Schiff base synthesis was applied to obtain the targeted azomethine.



Scheme 2. Synthesis of a new Schiff base

0.43 mmol of 5-bromo-2-hydroxy-3-methoxy, previously dissolved in 5 ml of ethanol, is placed in a two-necked flask equipped with a magnetic stirrer, magnet and reflux condenser, and heating is set. Then, 0.43 mmol of amine, dissolved in 5 ml of ethanol, is added to the flask while stirring. Upon addition of the amine, the color immediately changes from yellow to orange. After two hours, the solution in the flask is poured into ice water, forming a precipitate. The resulting precipitate is filtered, washed with water, and oven-dried. Yield is 96%

After recrystallization in ethanol, thin, long orange crystals of the final product form. Yield 91%. Melting point 151-153°C.

^{1}H NMR spectrum (Figure 3): (DMSO-d6, δ, ppm), 3.79 s (3H, OCH_3), 3.84 s (3H, OCH_3), 6.90 – 7.53 m (6H, $C_{Ar}H$), 8.89 s (1H, CHN), 13.53 s (1H, OH).

^{13}C NMR spectrum (Figure 4): (DMSO-d6, δ, ppm), 55.87 (OCH_3), 56.71 (OCH_3), 109.57 ($C_{Ar}H$), 115,16 ($C_{Ar}H$), 117,80 (C_{Ar}), 120.83 ($C_{Ar}H$), 123.13(CArH), 125.52 (C_{Ar}), 140.59 (C_{Ar}), 149.56 (C_{Ar}), 150.37 (C_{Ar}), 159.23 (C_{Ar}), 160.32 (CHN).

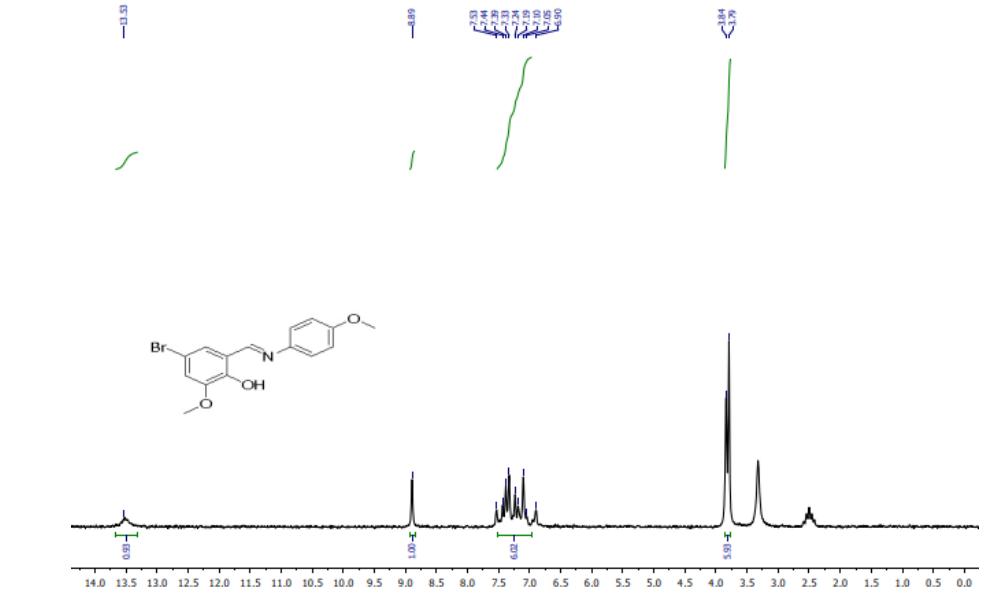


Figure 3. ^{1}H NMR spectra of the new Schiff base

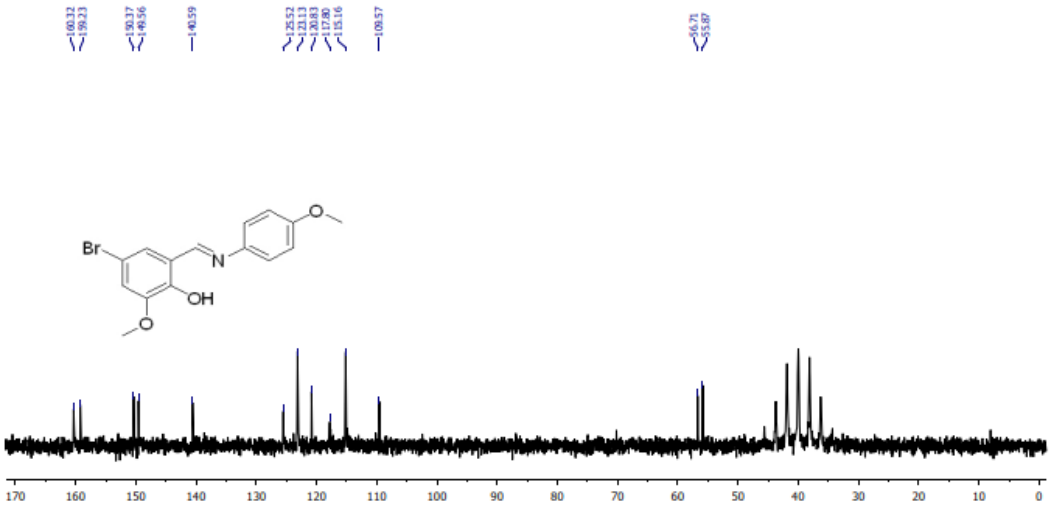


Figure 4. ^{13}C NMR spectrum of the new Schiff base

Nmr experiments

The NMR studies were carried out using Bruker Standard software (TopSpin 3.1) on a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany) (300 MHz for ^{1}H and 75 MHz for ^{13}C) with a BVT 3200 variable temperature unit in 5 mm sample tubes.

Tetramethylsilane (TMS) was the internal standard, and chemical changes were expressed in parts per million (ppm). The following multiplicities are identified: singlet (s), doublet (d), triplet (t), quadruplet (q), and multiple (m).

The experimental parameters for ^{1}H are follows: digital resolution=0.23 Hz, SWH=7530 Hz, TD=32 K, SI=16 K, 90º pulse-length=10 ms, PL1=3 dB, ns=1, ds=0, d1=1 s and for ^{13}C as follows: digital resolution=0.27 z, SWH=17985 Hz, TD=64 K, SI=32 K, 90º pulse length=9 ms, PL1=1.5 dB, ns=100, ds=2, d1=3 s. The NMR-grade DMSO-d6 (99.7%, containing 0.3% H2O) and $CDCl_3$ (99.8%, contains 0.5 wt. % silver foil as stabilizer) were used for the solutions of synthesized compounds.

Antimicrobial activity

A 96-well microtiter assay was used to evaluate the antimicrobial activity of the synthesized Schiff base against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The bacterial strains employed in the microbiological research were from the department of microbiology's culture collection at Baku State University in Azerbaijan. The inoculation of the new colony was done using Muller Hinton media (also known as "Liofilchem"). Various quantities of the evaluated compounds, ranging from 1024 to 8 μg/mL, were added to each well of the U-bottom microtiter, and then 105 CFU of various bacterial strains were added to each well. After a 24-hour period of incubation at 370C, the resazurin dye was employed to measure the amount of bacterial growth.

RESULTS AND DISCUSSIONS

The first step involves the synthesis of a primary aldehyde based on o-vanillin. Bromination of o-vanillin was carried out in acetic acid. Using acetic acid as a solvent and catalyst increased the yield of the reaction product. Since bromine is a pharmacophore, introducing a bromine atom into the aromatic ring enhances the biological activity of the Schiff base.

As can be seen from the hydrogen spectrum of the synthesized aldehyde (Figure 1) in the range of 7.16-7.31 ppm, which refers to the hydrogen signals of the aromatic ring, there are two doublet signals corresponding to two hydrogen atoms, which is confirmed by integration.

In the next step, a new Schiff base is synthesized from the resulting aldehyde. The absence of additional purification methods, other than washing with distilled water, is a particularly economically and environmentally advantageous aspect of using Schiff bases.

The ^{1}H NMR spectrum of the resulting new Schiff base (Figure 3) shows the disappearance of the signal at 9.85 ppm, corresponding to the aldehyde. A signal at 8.89 ppm appears instead, corresponding to the hydrogen in the imine bond. Also visible is a shift in the hydroxyl group signal from 11.00 ppm to 13.53 ppm, along with a visible broadening of the signal. The appearance of a second methoxy group in the hydrogen spectrum also confirms the formation of a Schiff base.

The ^{13}C NMR spectrum of the new Schiff base (Figure 4) clearly shows the absence of the aldehyde (the characteristic aldehyde signal at 195.41 ppm is absent) and the formation of the product (the signal at 160.32 ppm, characteristic of imines). The combined number of signals in the hydrogen and carbon spectra is consistent with the structure of the target product.

Table 1. Antibacterial activity analysis

Sample	S. aureus	E. coli	P. aeruginosa
Schiff base	64	32	128
Ampicillin	32	64	128

The biological activity of the resulting Schiff base was studied against a range of Gram-negative and Gram-positive bacteria, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The biological analysis results are presented in Table 1. As can be seen from the table, the tests demonstrate that the resulting Schiff base possesses antibacterial properties comparable to those of standard ampicillin. For example, against Escherichia coli, the Schiff base begins to exhibit activity at 32 μg/mL, while ampicillin only begins to act at 64 μg/mL. Conversely, the Schiff base's activity against Staphylococcus aureus is half that of the standard: 64 μg/mL and 32 μg/mL for the Schiff base and ampicillin, respectively. In the case of Pseudomonas aeruginosa, the indicators are comparable and both compounds begin to exhibit biological activity only at a minimum concentration of 128 μg/mL.

CONCLUSION

A new bromine-containing Schiff base derived from o-vanillin was successfully synthesized in a two-step process. In the first step, a 5-bromo derivative of o-vanillin is synthesized. The use of acetic acid as a solvent and catalyst increases the reaction yield and facilitates the bromination step. The introduction of bromine, known for its pharmacophoric properties, was aimed at enhancing the potential biological activity of the resulting compound.

The NMR spectroscopy data confirmed the successful formation of the Schiff base: the characteristic signal of the aldehyde proton disappeared from the hydrogen and carbon spectra, and new signals corresponding to the hydrogen and carbon in the imine bond (–CH=N–) appeared. Furthermore, the presence of a second methoxy group indicated a structural modification consistent with the expected product and confirmed the absence of unreacted aldehyde. The combined number of signals and protons confirms the structure of the resulting compound.

The synthesized Schiff base exhibited measurable antibacterial activity against both Gram-positive and Gram-negative bacterial strains, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The compound demonstrated activity comparable to the reference antibiotic ampicillin, showing enhanced inhibition against E. coli and moderate effects against S. aureus and P. aeruginosa.

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