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SYNTHESIS AND STRUCTURE OF VANILLIN AZOMETHINES*

*Vanillin is most prominent as the principal flavor and aroma compound in vanilla. Vanillin has been used as a chemical intermediate in the production of pharmaceuticals and other fine chemicals. The two intermediates, assigned as intermediates I and II, were prepared by using vanillin and 1-butybromide or 2-chloroacetic acid, respectively. The condensation of intermediates with 2,3-diaminopyridine gave azomethines. Their structure was established by using elemental microanalysis, as well as UV/Vis, FTIR and ¹H-NMR spectra. Synthesized compounds were tested for their antifungal activity against *Candida albicans*, *Candida lipolytica* and *Sacharomyces cerevisiae*.*

Key words: vanillin; azomethine; spectral analysis; antifungal activity.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla which is one of the most widely used and important flavoring materials worldwide [1-3]. Synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavoring in pharmaceutical preparations. Currently, approximately 50% of the worldwide production of synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the production of herbicides, antifoaming agents or drugs such as papaverine, l-dopa, l-methyldopa and the antimicrobial agent, trimethoprim [4]. Azomethines are of considerable interest because of their chemistry and potentially beneficial biological activities, such as antitumor, antibacterial, antiviral and antimalarial activities [5,6]. Considering all these, it was of importance to synthesized vanillin azomethines with a possible antimicrobial activity.

MATERIALS AND METHODS

Microanalysis for carbon, hydrogen and nitrogen was carried out with Carlo Erba 1106 microanalyser.

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The Fourier transform infrared spectra (FTIR) were recorded with a Bomem Hartmann & Braun MB-series spectrophotometer, using the potassium bromide KBr pellet (1 mg/100 mg) technique. The electronic spectra (UV/Vis) were recorded on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer using 10⁻³ mol·dm⁻³ solutions in DMF. The nuclear magnetic resonance (¹H-NMR) spectra were obtained in DMSO solution with a Gemini-200 “HF NMR”.

The antimicrobial screening was performed by the agar diffusion method using a paper disc. The sterilized agar (autoclaved at 120 °C for 30 min) was inoculated (1cm³/100 cm³ medium) with the suspension of microorganisms (matched to a McFarland barium sulphate standard) and poured into a Petri dish. The paper discs impregnated with synthesized azomethines (500 mg·cm⁻³) in *N,N*-dimethylformamide (DMF) were placed on the solidified medium. The zones of inhibition were measured after 72 h of incubation at 37 °C [7-9].

Preparation of 3-methoxy-4-butoxybenzaldehyde (intermediate I) and 3-methoxy-4-carboxymethylbenzaldehyde (intermediate II)

Intermediates I and II were synthesized using the procedure published by Wanhren *et al.* [10]. The obtained spectral data are in accordance with the spectral data already published by Wahren *et al.* [10].

Intermediate I. FTIR (KBr, (cm⁻¹)): 3100 (Ar-H), 1695 (C=O), 1355 (-CH₃), 1270, 1245, 1165, 1130,

1030 (C-O-C); UV/Vis (λ_{\max} , nm/ $\epsilon_{\max} \times 10^{-3}$, mol⁻¹ dm³ cm⁻¹): 315.0/1.1800 ($n \rightarrow \pi^*$), 273.0/1.3125 ($\pi \rightarrow \pi^*$), 230.0/0.9121 ($\pi \rightarrow \pi^*$), 201.0/1.6000. ¹H-NMR (200 MHz, DMSO-*d*₆, δ /ppm): 9.79 (*s*, 1H, -CH=O), 6.99–7.57 (*m*, 3H, Ar-H), 3.85 (*s*, 3H, -CH₃), 4.05–4.10 (*t*, 2H, -OCH₂-), 1.85 (*m*, 2H, -CH₂-), 1.50 (*m*, 2H, -CH₂-), 0.98 (*m*, 3H, -CH₃). Anal. Calcd.: C 69.23; H 7.69; Found: C 69.20; H 7.67; yield: 85.9%.

Intermediate II. FTIR (KBr, (cm⁻¹)): 2950 (-COOH), 1700 (C=O), 1355 (-CH₃), 1270, 1223, 1140, 1130 (C-O-C); UV/Vis (λ_{\max} , nm/ $\epsilon_{\max} \times 10^{-3}$, mol⁻¹·dm³·cm⁻¹): 310.0/0.9800 ($n \rightarrow \pi^*$), 270.0/1.3245 ($\pi \rightarrow \pi^*$), 228.0/1.9121 ($\pi \rightarrow \pi^*$), 201.0/1.5845. ¹H-NMR (200 MHz, DMSO-*d*₆, δ /ppm): 13.79 (*s*, 1H, -COOH), 9.70 (*s*, 1H, -CH=O), 7.02–7.65 (*m*, 3H, Ar-H), 4.56 (*s*, 2H, -CH₂-), 3.89 (*s*, 3H, -CH₃). Anal. Calcd.: C 57.14; H 4.76; Found: C 57.16; H 4.77; yield: 91.5%.

Preparation of azomethine I

2,3-*bis*(3-methoxy-4-butoxybenzaldehyde)di-amino pyridine (azomethine I) was prepared by the reaction of 3-methoxy-4-butoxybenzaldehyde (0.010 mol) and 2,3-diaminopyridine (0.0050 mol) in ethanol under reflux for 3 h (Figure 1). The precipitated product was filtered and recrystallised from the ethanol and dried in vacuo over CaCl₂ [11,12].

FTIR (KBr, (cm⁻¹)): >3000 (Ar-H), 1605 (HC=N), UV/Vis (λ_{\max} , nm/ $\epsilon_{\max} \times 10^{-3}$, mol⁻¹·dm³·cm⁻¹): 335.0/1.002 ($n \rightarrow \pi^*$), 283.0/1.120 ($\pi \rightarrow \pi^*$), 225.0/0.900 ($\pi \rightarrow \pi^*$), 207.0/1.145. ¹H-NMR (200 MHz, DMSO-*d*₆, δ /ppm): 9.51 (*s*, 1H, -CH=N), 8.95 (*s*, 1H, -CH=N), 8.43 (*s*, 1H), 7.92 (*s*, 1H), 7.49 (*t*, 1H), 7.39 (*t*, 1H), 7.32 (*t*,

1H), 7.15 (*t*, 2H), 6.95 (*m*, 2H), 3.85 (*s*, 3H, -CH₃), 4.10–4.15 (*t*, 2H, -OCH₂-), 1.97 (*m*, 2H, -CH₂-), 1.57 (*m*, 2H, -CH₂-), 1.04 (*t*, 3H, -CH₃). Anal. Calcd.: C 72.59; H 6.76; N 14.95; Found: C 72.55; H 6.75; N 14.91.

Preparation of azomethine II

2,3-*bis*(3-methoxy-4-carboxymethylbenzaldehyde)di-amino pyridine (azomethine II) was prepared by the reaction of 3-methoxy-4-carboxymethylbenzaldehyde (0.010 mol) and 2,3-diaminopyridine (0.0050 mol) in ethanol under reflux for 3 h (Figure 1). The precipitated product was filtered and recrystallised from the ethanol and dried in vacuo over CaCl₂ [9,11].

FTIR (KBr, (cm⁻¹)): 2980 (-COOH), 1602 (HC=N); UV/Vis (λ_{\max} , nm/ $\epsilon_{\max} \times 10^{-3}$, mol⁻¹·dm³·cm⁻¹): 320.0/0.9950 ($n \rightarrow \pi^*$), 268.0/1.1025 ($\pi \rightarrow \pi^*$), 235.0/1.4512 ($\pi \rightarrow \pi^*$), 210.0/1.5410. ¹H-NMR (200 MHz, DMSO-*d*₆, δ /ppm): 13.85 (*s*, 1H, -COOH), 9.55 (*s*, 1H, -CH=N), 8.99 (*s*, 1H, -CH=N), 8.46 (*s*, 1H), 7.95 (*s*, 1H), 7.45 (*t*, 1H), 7.38 (*t*, 1H), 7.34 (*t*, 1H), 7.16 (*t*, 2H), 6.95 (*m*, 2H), 3.85 (*s*, 3H, -CH₃), 4.10–4.15 (*t*, 2H, -OCH₂-), 1.97 (*m*, 2H, -CH₂-), 1.57 (*m*, 2H, -CH₂-), 1.04 (*t*, 3H, -CH₃). Anal. Calcd.: C 63.60; H 4.59; N 14.84; Found: C 63.59; H 4.62; N 14.81.

RESULTS AND DISCUSSION

All compounds were isolated in high yields and are air-stable both in the solid-state and in solution. The results of elemental analysis are consistent with the proposed structure of the obtained intermediates and azomethines (Figure 1).

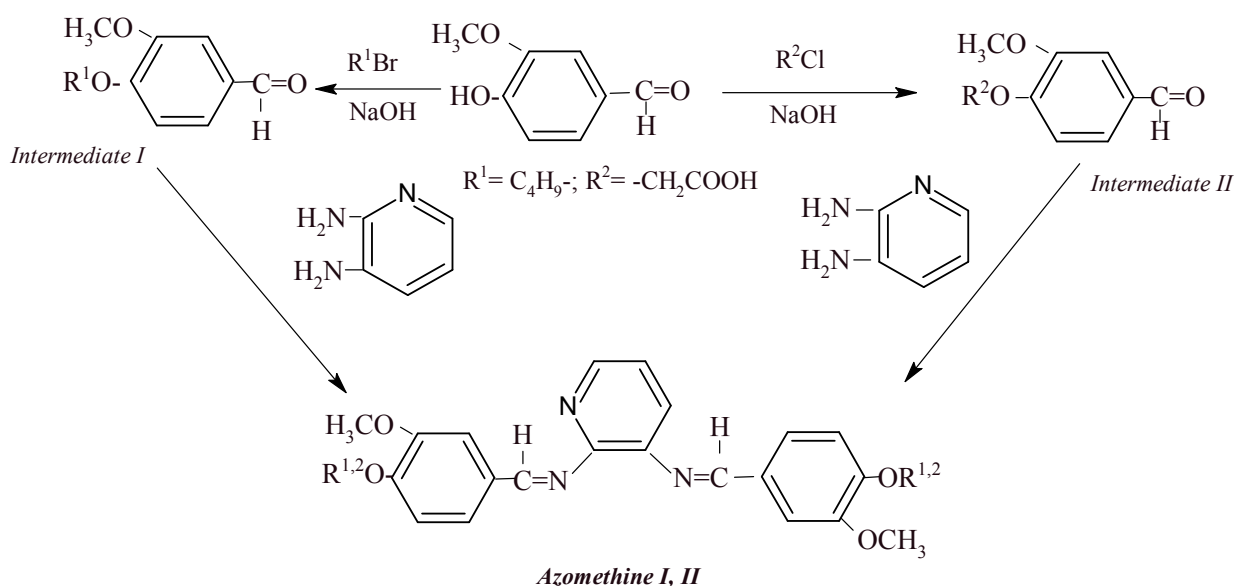


Figure 1. Synthesis of azomethines.

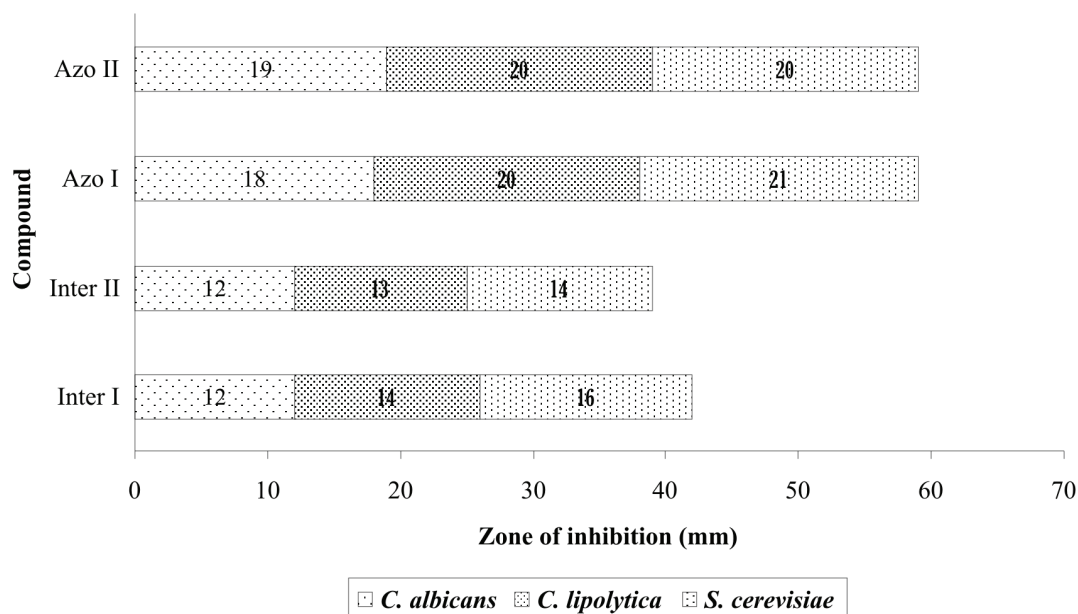


Figure 2. Antifungal activity of vanillin azomethines.

The spectral data of the intermediates shows absorption bands, assigned to $n-\pi^*$ and $\pi-\pi^*$ transitions. These transitions are also found in the spectra of azomethines, but they are shifted towards lower and higher frequencies, confirming the formation of these compounds. The characteristics of the FTIR spectra of all complexes are in agreement with the suggested structural formulae. The bands corresponding to $\nu(\text{C}=\text{O})$ are present in the spectra of intermediates I and II (1695 and 1700 cm^{-1} , respectively). The disappearance of the $\nu(\text{C}=\text{O})$ bond in the spectra of azomethines, and the appearance of a new band at 1605 and 1602 cm^{-1} in the spectra of azomethines, suggest the formation of new compounds. In the spectra of the intermediates I and II, the $\text{HC}=\text{O}$ resonance signal is observed as a singlet at 9.79 and 9.70 ppm, respectively. This signal is not found in the spectra of azomethines but a signal of a $\text{HC}=\text{N}$ proton appears in the spectra of both azomethines. Thus, the appearance of the azomethine proton signal and the disappearance of the $\text{HC}=\text{O}$ group resonance signal both suggest the condensation of the intermediates and 2,3-diaminopyridine.

The vanilla derivatives were evaluated for their *in vitro* antifungal activity against *Candida albicans*, *Candida lipolytica* and *Sacharomyces cerevisiae* (Figure 2). According to the obtained results, azomethines show the enhanced activity compared to the intermediates. This was expected considering the fact that antifungal activity of vanillin derivatives is enhanced due to the antimicrobial active $\text{C}=\text{N}$ group [6]. All synthesized compounds have the largest antifungal activity against *Sacharomyces cerevisiae*.

CONCLUSION

Vanillin azomethines have been synthesized and characterized. The antifungal activity of the obtained compounds show that vanillin azomethines have enhanced the activity more than intermediates due to $\text{C}=\text{N}$ group.

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