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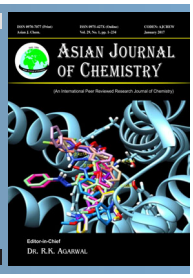

Syntheses of Novel Pyrazolines as Antibacterial Agents from Natural Product Vanillin

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A series of pyrazoline derivatives have been synthesized from natural vanillin and tested as antibacterial agents by diffusion method. Pyrazolines were synthesized from vanillin *via* chalcone intermediate. The structure of all the products was elucidated by FTIR, GC-MS, ^{1}H NMR and ^{13}C NMR spectrometers. All the tested compounds showed their potential as antibacterial agents in broad spectrum. Inhibition value of all the active compounds was compared with standard drug tetracycline and DMSO. The highest antibacterial activity of pyrazoline derivatives was found against *Staphylococcus aureus* (1000 ppm, 7.25 mm). Antibacterial tests of pyrazoline derivatives showed that *N*-acetyl pyrazoline increased the antibacterial activity at Gram-positive bacteria but decreased at Gram-negative ones.

Keywords: Vanillin, Chalcone, Pyrazoline, Antibacterial.

INTRODUCTION

Bacterial pathogens are the main cause of human disease in developing countries and can cause, for example, malaria, tuberculosis, cholera, pneumonia, respiratory problems and diarrhea [1]. Treatment of these infections became more difficult because the emergence of drug resistant strains [2,3].

Compounds with C=N and N-N groups, such as pyrazolines, have many pharmacological activities. Pyrazolines are well known five membered heterocyclic compounds with antibacterial properties [4,5]. In addition, pyrazoline derivative compounds have analgesic and anti-inflammatory properties [6].

Several methods exist for the synthesis of pyrazolines. One of the most important methods is the reaction of α,β-unsaturated ketones with 1,2-binucleophile by refluxing [7]. Several pyrazoline compounds have been modified to be more active as antibacterial agents by the addition of bromide [8,9], hydroxy and methoxy groups [10]. Designing and exploring a new a synthetic method should consider the availability of materials. Vanillin and wintergreen oil are an aldehyde source from available natural resources.

Several studies focused on the influence of substituents on the phenyl group of pyrazoline on its antibacterial ability. But the effect of substituents on *N*-pyrazoline has not been widely reported. This research focuses on the syntheses of *N*-acetylated pyrazoline derived from vanillin and wintergreen oil and testing of the antibacterial properties of this compound.

EXPERIMENTAL

All chemicals were procured from E. Merck with pro-analysis (p.a) quality such as vanillin, 2-hydroxycetophenone, hydrazine monohydrate, potassium bromate, sodium thiosulphate, sodium hydroxide, hydrochloric acid, glacial acetic acid, ethanol, methanol, *n*-hexane, ethyl acetate and dimethyl sulfoxide 99.9 %.

Melting points were measured using Electrothermal 9100 melting point apparatus and are uncorrected. Infrared spectra were recorded with a Shimadzu Prestige-21 FTIR spectrometer using KBr disc. ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL JNMECA (500 MHz) spectrometer. Mass spectra were recorded on Agilent GC-6890 and MS-5973 (EI). The purity of the compounds was confirmed by GC and thin layer chromatography.

3-Bromo-4-hydroxy-5-methoxybenzaldehyde (1): Vanillin was brominated by Schatz method [11] under acidic condition using $KBrO_3$ as bromide source and CH_3COOH as acid. The white crystal was yielded (98 %) with m.p.: 164 °C, IR spectrum (KBr, cm^{-1}): 3333 (OH), 2846 and 2746 (C-H), 1674 (C=O), 1589 (C=C), 1357 (C-H). ^{1}H NMR spectrum (500 MHz, $CDCl_3$): δ 3.90 (s, 3H, OCH_3), 7.41 (d, 1H, J = 1.3 Hz, Ar-H), 7.72 (d, 1H, J = 1.3 Hz, CH_α), 9.77 (s, 1H, CHO). ^{13}C NMR spectrum (125 MHz, $CDCl_3$): δ 29.88 (OCH_3), 56.73-149.78 (Ar-C), 190.43 (CHO. MS: 232 (M+2).

(*E*)-3-(3-Bromo-4-hydroxy-5-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (2): 5-Bromovanillin (1.07 g,

5 mmol) was dissolved in methanol and solution of 2-hydroxy-acetophenone (0.67 mL, 5 mmol) in 12 mL NaOH 20 % was added. The mixture was refluxed for 24 h then poured into crushed ice and acidified with HCl 10 % to form a precipitate. The precipitate was filtered off and recrystallized from ethanol to give yellow solid crystal. Yield 78 %, m.p. 168 °C, IR spectrum (KBr, cm^{-1}): 3387 (OH), 1635 (C=O), 979 (C-H *trans*-bend). ^{1}H NMR spectrum (500 MHz, $CDCl_3$): δ 3.99 (s, 3H, CH_3), 6.22 (s, 1H, OH), 7.5 (d, 1H, *J* = 14, 95 Hz, CH_α), 7.79 (d, 1H, *J* = 15.55 Hz, CH_β). ^{13}C NMR spectrum (125 MHz, $CDCl_3$): δ 29.88 (CH_3), 109.05 (C-Br), 144.37 & 163.78 (C-OH), 193.50 (C=O). MS: 350 (M+2).

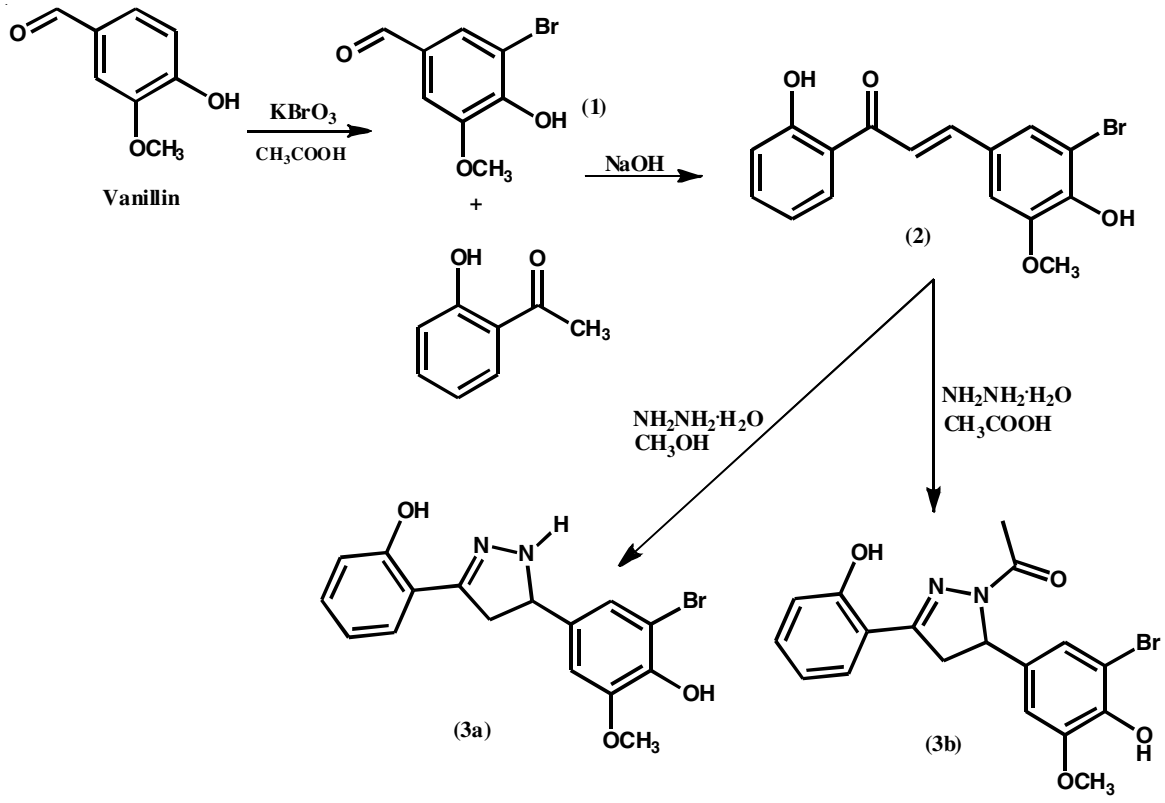
2-Bromo-4-[3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl]-6-methoxyphenol (3a): Chalcone (**2**) (0.35 g, 1 mmol) was dissolved in methanol (10 mL) to which hydrazine monohydrate (0.1 mL, 2 mmol) was added. The mixture was refluxed for 24 h, then poured onto crushed ice and allowed to stand in the refrigerator for over night. The precipitate was filtered off, washed with cold water and dried to get grey powder (**Scheme-I**). Yield 87.04 %, m.p. 251 °C, IR (KBr, cm^{-1}) : 3333 (NH), 1589 (C=N), ^{1}H NMR (500 MHz, $CDCl_3$): δ 3,07 (dod,1H, *J* = 7.15; 9.57 Hz, CH), 3.56 (dod, 1H, *J* = 5.85; 11 Hz, CH), 3.92 (d, 1H, CH_3), 4.81 (t, 1H, CH), 5.95 (d, 1H, NH), 7.10 and 10.94 (s, 1H, OH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 42.11 (CH_2), 56.60 (C-N), 62.49 (C-O-C), 147.71 (C=N). GC 87.04 %, MS: 364 (M+2).

1-[5-(3-Bromo-4-hydroxy-5-methoxyphenyl)-3-(2-hydroxyphenyl]-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (3b): Chalcone (**2**) (0.35 g, 1 mmol) was dissolved in glacial acetic acid (6 mL) and hydrazine monohydrate (0.1 mL, 2 mmol) was added. The mixture was heated to 60-70 °C and stirred for 6 h then poured onto crushed ice. The precipitate was filtered off, washed with ethanol and dried to get white powder (**Scheme-I**). Yield 88.88 %, m.p. 106-111 °C, IR (KBr, cm^{-1}): 3425 (OH), 2924 (CH_3-sym), 2854 (CH_3-asym), 1643 (C=O), 1597 (C=N), ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3), 3.28 *J* = 17.82 Hz; 5.2 (dod, 1H, CH), 3.85 *J* = 17.82 Hz; 11,7 (dod, 1H, CH), 5.44 *J* = 12.05 Hz; 5.2 (dod, 1H, CH), ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.88 (CH_3), 42.91 (CH), 56.60 (C-N), 58.47 (C-O-C), 147.51 (C=N). GC 90 %, MS: 406 (M+2).

RESULTS AND DISCUSSION

The pyrazolines derivatives were screened for the anti-bacterial activity against five bacterial strains, *i.e.* three Gram-positive bacteria (*Staphylococcus aureus* FNCC 0047, *Bacillus substilis* FNCC 0041 and *Bacillus cereus* FNCC 0040) and two Gram-negative bacteria (*Eschericia coli* FNCC 0091 and *Shigella flexnerri* ATCC 12022). Tetracycline (100 ppm/disc) was used as standard antibiotic references and dimethyl sulfoxide (DMSO) was used as negative control for the comparison purpose. After incubation the diameter of zone of inhibition formed around the cavities and disc of standard drugs was accurately measured in mm. The observed zones of inhibition are presented in Table-1.

According to these results, pyrazoline showed a weak to moderate activity. Pyrazoline compounds **3a** and **3b** have the highest activity against *Bacillus cereus* (1000 ppm: 6.1 mm); *Staphylococcus aureus* (1000 ppm: 7.25 mm) and *Bacillus subtilis* (1000 ppm: 6.95 mm). It was observed that change of hydrogen atom into acetyl group at *N*-pyrazoline increased



Scheme-I: Synthesis of pyrazolines (**3a** and **3b**)

TABLE-1
ANTIBACTERIAL DATA OF PYRAZOLINE DERIVATIVES (**3a** and **3b**)

Conc. (ppm)	Zone of inhibition (mm)									
	Compound **3a**					Compound **3b**				
	Gram-positive			Gram-negative		Gram-positive			Gram-negative	
	S. aureus	*B. subtilis*	*B. cereus*	*E. coli*	*S. flexnery*	*S. aureus*	*B. subtilis*	*B. cereus*	*E. coli*	*S. flexnery*
100	2.40	5.37	5.50	3.37	2.55	3.25	4.67	5.00	–	2.75
300	4.00	3.75	4.42	3.00	3.25	4.45	6.65	5.45	–	2.75
500	3.45	5.65	5.50	1.25	3.35	6.60	6.77	5.00	–	–
1000	4.77	5.63	6.10	2.40	4.58	7.25	6.95	5.50	–	–
Control (+)	16.37	12.67	11.03	15.33	15.12	16.00	12.8	11.68	16.35	15.05
Control (–)	–	–	–	–	–	–	–	–	–	–

the antibacterial activity at Gram-positive bacteria. In contrast, antibacterial activity at Gram-negative was decreased.

Conclusion

In the present study, two pyrazolines (**3a** and **3b**) have been synthesized and yielded in 87.04 and 88.83 %, respectively. Antibacterial test of pyrazoline derivatives showed that *N*-acetyl pyrazoline increased the antibacterial activity at Gram-positive but decreased at Gram-negative. The highest antibacterial activity of each pyrazoline (**3a**) and pyrazoline (**3b**) were to against *Bacillus cereus* (1000 ppm: 6.1 mm); *Staphylococcus aureus* (1000 ppm: 7.25 mm) and *Bacillus subtilis* (1000 ppm: 6.95 mm).

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