

Synthesis of 3-propyl-5-pyrazolone

Compound	(1): M ₃ PzO 3-methyl-5-pyrazolone	(2): P ₃ PzO 3-propyl-5-pyrazolone	(3): PhPzO 3-phenyl-5-pyrazolone
Structure			

BJP

Bangladesh Journal of Pharmacology

Research Article

***In vitro* antimicrobial evaluation of pyrazolone derivatives**

***In vitro* antimicrobial evaluation of pyrazolone derivatives**

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Article Info

Received: 8 December 2014
Accepted: 17 January 2015
Available Online: 22 February 2015
DOI: 10.3329/bjp.v10i1.21166

Cite this article:

Ahar LR, Sadr MH, Zare K, Tabatabaei SM. *In vitro* antimicrobial evaluation of pyrazolone derivatives. Bangladesh J Pharmacol. 2015; 10: 143-47.

Abstract

In this investigation, unsubstituted pyrazolin-3(5)-ones (MePzO= 3-methyl-5-pyrazolone, PrPzO= 3-propyl-5-pyrazolone and PhPzO= 3-phenyl-5-pyrazolone) have been synthesized. The synthesized compounds were also screened for their antibacterial activity against some Gram-positive and Gram-negative bacteria using agar well diffusion method. The compounds with different concentrations were prepared and the activity was determined by measuring the diameter of the inhibition zone (in mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the compounds were determined against six bacteria. The antibacterial activities were influenced functional groups (methyl, propyl and phenyl). PhPzO exhibited the strongest activity (MIC, 0.625 mg/mL; MBC, 2.5 mg/mL) against *Bacillus subtilis*.

Introduction

Pyrazolin-5-ones are well known nitrogen containing five membered heterocyclic compounds. They have been used as therapeutic agents, such as analgesic, antipyretic, anti-inflammatory (Brogden, 1986; Gursoy et al., 2000; Ratnadeep et al., 2010; El-Hawash et al., 2006; Chao et al., 2008), antioxidant (Manojkumar et al., 2009; Watanabe et al., 1994; Ramana Kumar et al., 2012), anti-proliferative (Kim et al., 2005), antibacterial (Al-Haiza et al., 2001; Raman et al., 2004), antifungal activities (Vincent, 1999; Ramaraj et al., 2010) and is useful in the treatment of a variety of disorders caused by Human Immunodeficiency Virus (HIV) (Hadi et al., 2010). Pyrazolone derivatives, such as antipyrine (phenazone), 4-aminopyrine (aminophenazone), metamizol (novalgine), and 4-isopropylpyrine (propyphenazone; PP) are analgesic substances (Himly et al., 2003) and edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is found as a drug for brain ischemia, myocardial ischemia, treatment of fatal neurodegenerative diseases and cardiovascular diseases (Tsujita et al., 2004; Higashi et al., 2006; Zhang et al., 2012; Kikuchi et al., 2010).

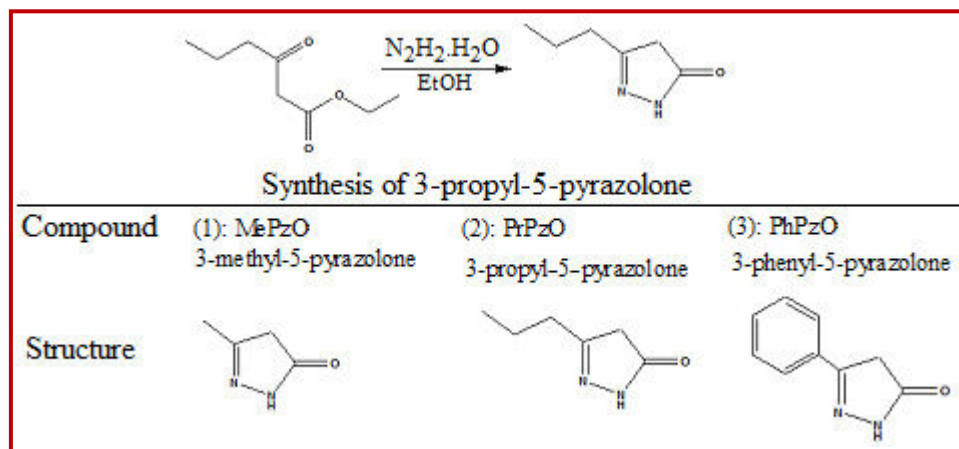
In view of these observations, we report the synthesis of

pyrazolone derivatives, and their antimicrobial evaluation and investigate the effects of concentrations of the compounds on the bacterial strains. The derivatives show good inhibition towards some bacteria.

Materials and Methods

As a part of this study for vibrational spectra, we analyzed the infrared spectra recorded on a FT Bruker Vector 22 infrared spectrophotometer using pressed KBr disk. The melting point was determined using a Barnstead (BI 9300) electrothermal apparatus. Starting materials ethyl acetoacetate, ethyl butyrylacetate and ethyl benzoylacetate were purchased from Acros. Hydrazine monohydrate and ethanol were purchased from Merck. The solvent was dried before use by the standard methods (Perrin, 1988). The preparation of pyrazolone derivatives involve the condensation of a β -keto ester with substituted or unsubstituted hydrazines. The compounds were synthesized via a similar method described elsewhere (Khalil et al., 2005; Makhija et al., 2004; Song and Zhu, 2001; Abood and Al-Shilhai, 2012). The synthesis of 3-propyl-5-pyrazolone and the structures of the compounds in this investigation are shown





Scheme 1: The synthesis of 3-propyl-5-pyrazolone and the structures of the compounds

in **Scheme 1**.

Synthesis of the 3-methyl-5-pyrazolone (1), 3-propyl-5-pyrazolone (2) and 3-phenyl-5-pyrazolone (3): 3-Methyl-5-pyrazolone [from ethyl acetoacetate with hydrazine monohydrate in hot ethanol, Yield: 82%. m.p.: 202-203 °C; FTIR (KBr, cm⁻¹): 3400 (νN-H), 2300-3242 (νC-H, m), 1678 (νC=O, s), 1609 (νC=N, m), 956 (N-N, w)]; 3-propyl-5-pyrazolone [from ethyl butyrylacetate with hydrazine monohydrate in hot ethanol, Yield: 88 %; m.p.: 207-209 °C; FTIR (KBr, cm⁻¹): 3165 (νN-H), 2344-2959 (νC-H, m), 1620 (νC=O, s), 1599 (νC=N, m), 901 (N-N, w)]; and 3-phenyl-5-pyrazolone [from ethyl benzoylacetate with hydrazine monohydrate in hot ethanol, Yield: 90%; m.p.: 240-241 °C; FTIR (KBr, cm⁻¹): 3448 (νN-H), 2300-3121 (νC-H, m), 1624 (νC=O, s), 1491 (νC=N, m), 930 (N-N, w)] were prepared.

Antimicrobial assay

The antibacterial test of the synthe-sized compounds (1, 2 and 3) was carried out using the agar well diffusion method (Chohan et al., 2003) to determine the

minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

The synthesized compounds were screened for their antibacterial activities against six bacterial strains of three Gram-negative bacteria *Escherichia coli* (PTCC 1270), *Salmonella enterica* (PTCC 1709), *Pseudomonas aeruginosa* (PTCC 1430) and three Gram-positive bacteria *Staphylococcus aureus* (PTCC 1112), *Enterococcus faecalis* (PTCC 1237), *Bacillus subtilis* (PTCC 1254) by the agar well diffusion method (Table I) (Chohan et al., 2003). Tetracycline (TE 30), was used as standard for antibacterial activity. All the microbial cultures were adjusted to 0.5 Mc Farland standards (equivalent turbidity 1/5 × 10⁸ CFU/mL). 20 mL of Mueller Hinton Agar medium was poured into each petri plate (12 cm) and plates were swabbed with 100 μL inocula of the test microorganisms and kept for 15 min for adsorption. The compounds to be tested for activity were dissolved in dimethylsulfoxide. In the first step, ten milligrams of the compounds were dissolved in 1 mL of DMSO. Using sterile cork borer of 6 mm diameter, wells were

Table I

Inhibition of bacterial growth by using different concentration of the compounds

Bacterium	<i>S. aureus</i>			<i>E. faecalis</i>			<i>B. subtilis</i>		
Standard drug	25			25			25		
Concentration (mg/mL)	10	5	2.5 - 0.04	10	5	2.5-0.04	10	5	2.5-0.04
1	0	0	0	0	0	0	12	0	0
2	NT	13	0	NT	0	0	NT	0	0
3	13	0	0	0	0	0	0	0	0
Bacterium	<i>E. coli</i>			<i>S. enterica</i>			<i>P. aeruginosa</i>		
Standard drug	13			8			15		
Concentration (mg/mL)	10	5	2.5-0.04	10	5	2.5-0.04	10	5	2.5-0.04
1	0	0	0	0	0	0	8	0	0
2	NT	10	0	NT	0	0	NT	10	0
3	0	0	0	8	0	0	0	0	0

NT = Not tested; Tetracycline (TE 30) is used as standard

Table II

Antibacterial activity of the compounds by MIC and MBC tests

Bacteria	Minimum inhibitory concentrations (mg/mL)				Minimum bactericidal concentrations (mg/mL)			
	(1)	(2)	(3)	The order of activity	(1)	(2)	(3)	The order of activity
<i>S. aureus</i>	2.5	1.2	2.5	1=3<2	5	5	10	3<1=2
<i>E. faecalis</i>	2.5	2.5	2.5	1=2=3	10	>10	>10	3, 2<1
<i>B. subtilis</i>	1.2	1.2	0.6	1=2<3	5	5	2.5	1=2<3
<i>E. coli</i>	2.5	1.2	2.5	1=3<2	10	5	10	1=3<2
<i>S. enterica</i>	5	2.5	5	1=3<2	5	2.5	>10	3<1<2
<i>P. aeruginosa</i>	2.5	1.2	2.5	1=3<2	10	5	10	1=3<2

bored into the seeded agar plates and these were loaded with a 100 mL volume with concentration of 10.0-0.039 mg/mL. All the plates were incubated at 37°C for 24 hours and the inhibition zone diameters around each disk were measured and recorded in millimeters and presented in Table I. MIC values against these organisms were determined by serial dilution method using DMSO. MIC determination was performed in a 96-well micro-titer plate. For MIC assay, a row of 12 wells was used. In this method, the various test concentrations of synthesized compounds were prepared from 10 to 0.039 mg/mL (No. 1 to 9) and was labeled 10-0.039 mg/mL. 100 µL sterile Mueller Hinton Broth (MHB) was poured in the wells from 2-12 followed by addition of 100 µL test compound in well 1, 2 and 12 containing 10 mg/mL concentration. In this way a range of two-fold serial dilution were prepared (10-0.039 mg/mL) by performing two-fold serial dilution from well 2 to the well 9. To each well, 100 µL of standard inoculums (1.5×10^8 CFU/mL) was added from well 1 to the well 10. Then 10 µL resazurin was added in each of the wells. Turbidity was observed after incubating the inoculated wells at 37°C for 24 hours. The last dilution with no turbidity was considered as MIC. Then from all wells were cultured on plates to determine the minimum lethal bacterial concentration (MBC). Then plates were incubated at 37°C for 24 hours. The well containing the lowest concentration with lack of bacterial growth was visible as MBC. Both MIC and MBC results are presented in Table II.

Results and Discussion

The antibacterial activity and inhibition zone around tested compounds can be attributed to their bactericidal effect that is due to killing of bacteria or their bacteriostatic effect that inhibits bacterial growth and colony formation through blocking of active sites on surface or inside bacterial cell (Sedaghat et al., 2013). The MIC was defined as the lowest concentration of a substance that inhibited visible growth of the microorganisms. The MBC was the lowest concentration at which no colony

formulation was observed on the agar plates, determined by spreading 100 µL of each microorganism on a MHA plate. These plates were incubated at 37°C for 24 hours. The range of antimicrobial activity more than 30 mm shows potent activity, for 21-30 mm inhibition activity is strong activity, 16-20 mm is moderate activity, 10-15 mm is weak activity and below 10 mm is little or no activity (Jeon et al., 2014) or Inhibition zone more than 12 mm shows significant activity, for 10-12 mm inhibition activity is good, 7-9 mm is low, and below 7 mm is non-significant activity (Ahmad et al., 2008).

All the compounds were tested against Gram-positive and Gram-negative bacteria. The structure activity relationship analyses of the compounds were compared. The compounds were screened for *Staphylococcus aureus* and compounds (2) and (3) had lower activities than the standard tetracycline, and compound (1) exhibited no activity. The compounds (1-3) were screened for *Enterococcus faecalis*, and had no activity. The compounds were screened for *Bacillus subtilis* and compound (1) had low activity compared with the standard tetracycline and compounds (2) and (3) exhibited no activity. The compounds (1-3) were screened for *Escherichia coli* and compounds (1) and (3) exhibited no activity, while compound (2) was found to have low activity compared with the standard tetracycline. The compounds (1-3) were screened for *Salmonella enterica*, the compound (3) had an equipotent activity compared with the standard tetracycline, while compounds (1) and (2) had no activity. The compounds were screened for *Pseudomonas aeruginosa*, whereby the compound (1) was less active compared with the standard tetracycline, while the other compounds (2) and (3) had no activity.

The PhPzO (10 mg/mL) and PrPzO (5 mg/mL) exhibited the highest activity against *Staphylococcus aureus* with zone of inhibition 13 mm. The bacterial zones of inhibition values (mm) of the test compounds are given in Table I.

Table I shows that *S. aureus* strain is more susceptible to the inhibition growth effect of the compounds. MePzO

was found to show maximum activity against *B. subtilis* and *P. aeruginosa* at 10 mg/mL with zone of inhibition 12 and 8 mm, respectively. *S. aureus* and *S. enterica* showed maximum activity by PhPzO with zone of inhibition 13 and 8 mm, respectively.

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on the antibiotic-free media (Andrews, 2001). The MIC is a measure of the potency of an antimicrobial compounds. The highest MIC and MBC values of microorganisms are clear indications that compounds are less effective on some bacteria, while the low MIC and MBC values for bacteria are indications of the efficacy of the compounds. Results of MIC and MBC are shown in Table II.

The results show that the PhPzO, PrPzO and MePzO exhibit significantly enhanced activity toward all bacteria under study with MIC at range 0.625-5 mg/mL, 1.25-2.5 mg/mL and 1.25-5 mg/mL, respectively. The PrPzO has the highest activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* at 1.25 mg/mL and is least effective against *S. enterica* and *E. faecalis* at 2.5 mg/mL. *Salmonella enterica* is the least affected organism by PhPzO and MePzO at MIC 5 mg/mL; and *Bacillus subtilis* is most affected at MIC 0.625 mg/mL and 1.25 mg/mL, respectively.

The MBC of MePzO for *S. aureus*, *B. subtilis*, and *S. enterica* was 5 mg/mL and 10 mg/mL for *E. faecalis*, *E. coli* and *P. aeruginosa*.

The MBC of PrPzO and PhPzO for tested bacteria were in the range of 2.5 to >10 mg/mL. The result MBC of PrPzO and PhPzO showed the lowest MBC of 2.5 mg/mL were shown by *S. enterica* and *B. subtilis*. The order of activity for the studied compounds was shown in Table II.

In conclusion, the MIC and MBC values of the compounds indicated that compound (2) has the highest activity against Gram-negative bacteria.

Acknowledgment

The author is thankful for Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran for support of this work.

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