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Synthesis and characterisation of pyrazole derivatives from para bromo benzoic acid and its antibacterial activity

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Abstract
Pyrazole is an organic compound with the formula $C_3H_3N_2H$. It is a heterocycle characterized by a 5-membered ring of three carbon atoms and two adjacent nitrogen atoms. Numerous pyrazole derivatives have been found to possess a broad spectrum of biological activities like anti-bacterial, anti-convulsant, analgesic, anti-microbial, anti-inflammatory, anti-diabetic, sedative anti-rheumatic, anticancer, and anti-tubercular activities. The compounds were synthesized by the normal conventional technique in the lab. All the synthesized compounds were screened for antibacterial activities at three different concentrations (100µg/ml, 250µg/ml, 500µg/ml) against *Pseudomonas aeruginosa* (Gram –ve) and *Bacillus subtilis* (Gram + ve) by cup plate agar diffusion method and MIC was found out.

Keywords: pyrazole derivatives, antibacterial activity, MIC

1. Introduction
Pyrazole is a □-excessive heterocycle and contain 2 nitrogen atoms adjacent to each other. P-sulfamyl phenylhydrazine and chalcones were used for the synthesis of pyrazole derivatives such as 5-Anthracen-9-yl-3-aryl-1-(p-sulfamylphenyl) pyrazoles [1]. These synthesized pyrazole analogues were evaluated for antimicrobial activity showing prominent activity.The present study was aimed at synthesising some novel compounds and to screen the synthesized compounds for antibacterial activity.

2. Materials and Methods
2.1 All the materials used for the research activity were purchased from the NICE Pharmaceuticals, KOCHI and Hi media, Mumbai. The biological samples (*Bacillus subtilis* and *Pseudomonas aeuroginosa*) were obtained from DDRLC laboratories,Kottayam.

2.2. Methodology
The final product was made in a step wise manner.

2.2.1 Step 1: Synthesis of Ethyl-4-bromo benzoate [3]
P-bromo benzoic acid(30g) in ethanol was added with 150ml con.sulphuric acid at 0.5ºC over a period of 30min and refluxed for 2hrs on a water bath. The reaction mixture was poured into ice-cold water. The solid thus obtained was filtered, washed and dried. The dried product was recrystallised from ethanol to white needle shaped crystals.

2.2.2 Step 2: Synthesis of 4-bromo benzohydrazide [4]
The mixture of 0.167 mol (29g) of substituted esters and 0.167 mol(5g) of hydrazine was warmed with 60ml ethanol and few drops of glacial acetic acid. The reaction mixture was cooled and filtered. The solid thus obtained was washed with dil.HCl followed by about 12ml of cold rectified spirit. The dried product was recrystallised from ethanol to white needle shaped crystals of pure 4-bromo benzohydrazide.

2.2.3 Step 3: Synthesis of (E)-4-bromo-N'-(1-(4-substituted phenyl) ethylidene) benzohydrazide [2, 5]
0.01 mol of substituted acetophenone (methoxy, methyl, bromo, chloro and fluoro) was added to the mixture containing 0.01 mol of 4-bromo benzohydraide in 30ml ethanol and few drops of glacial acetic acid. The reaction mixture was refluxed for 1hr and then cooled in an ice-bath. The product separated on cooling was filtered, dried, and recrystalised from ethanol to white needle like crystals.

2.2.4 Step 4: Synthesis of 1-(4-bromobenzoyl)-3-(4-substituted phenyl)-1H-pyrazole-4-carbaldehyde [3, 4]
Cyclisation: The substituted hydrazones (methoxy, methyl, bromo, chloroand fluoro)(0.005mol) were added into the mixture of Vilsmeier-Haack (DMF & POCl3) reagent, prepared by dropwise addition of phosphorous oxy chloride 140ml(0.015mol) to an ice-cold solution of N,N-dimethyl formamide 20ml. The reaction mixture was refluxed for 2hrs, then poured into ice-cold water and neutralized using an excess of sodium bicarbonate solution. The product was washed with water and recrystallised from ethanol.

2.3 *In vitro* Antibacterial Screening
The *in vitro* bacterial activity was evaluated by Cup plate agar diffusion method and Tube dilution method (turbidometric method). The turbidometric method depends upon the inhibition of growth of microbial culture in a uniform solution of antibacterial agent. In this method minimal inhibitory concentration (MIC) of the lowest concentration of an antibacterial agent that inhibits the growth of test organism can be detected. The suspension of all the organisms were prepared by inoculating one colony of the bacterial strain in 20ml of nutrient broth. The compounds were weighed and dissolved in Dimethyl sulphoxide to prepare appropriate dilution to get the required concentrations. A control was prepared by using DMSO alone.

3. Results & Discussions

Three compounds were synthesized and characterised using the physical as well as spectral datas.

3.1 Characterisation

The formation and purity of the synthesized compounds were ascertained by melting point and Rf values.

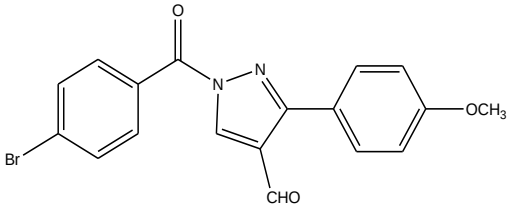
Table 1: Characterisation by melting point determination and TLC

Compound Code	R	MW (D)	M.P (^{0}C)	Rf	Solvent system
AV1	4-OCH_3	385.21	230-232^{0}C	0.67	Chloroform: Methanol (9:1)
AV2	4-CH_3	369.21	215-218^{0}C	0.72	Chloroform: Methanol (9:1)
AV3	4-Br	434.08	310-315^{0}C	0.60	Chloroform: Methanol (9:1)

3.2 Spectral analysis [6, 7, 8, 9]

The characterization of the derivatives were carried out by various spectroscopic methods such as IR, 1HNMR and MASS spectrometry.

Compound: AV1

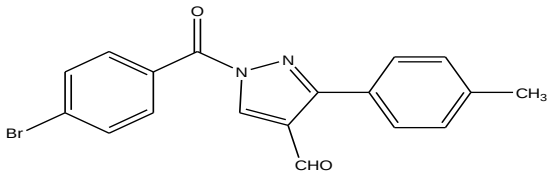


1-(4-bromobenzoyl)-3-(4-methoxy phenyl)-1H-pyrazole-4-carbaldehyde

Table 2: Spectral datas of AV1

Compound Code	IR	Streching	NMR	Interference	Mass
AV1	2922	Aromatic –CH stretching	7.5-7.9 – Multiplet	Ar-H	M^+ peak = 385.21
	2852	Aliphatic –CH- stretching	6.98 - Singlet	-N-CH-	
	1583	Aromatic C=C stretching	3.43 - Singlet	-OCH_3	
	927	Aromatic C-C stretching	9.98 - Singlet	- CHO	
	1323	-C-N- stretching			
	1127	-N-N=C stretching			Base peak = 98.06
	1679	C=O stretching			
	1298	-C-O-C stretching			
	755	-C-Br stretching			

Compound: AV2

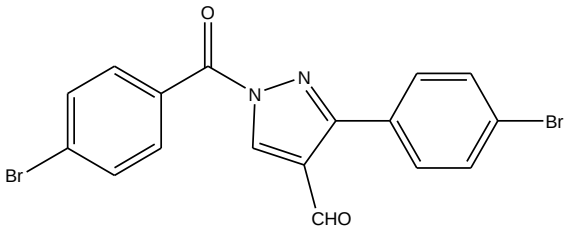


1-(4-bromobenzoyl)-3-(4-methyl phenyl)-1H-pyrazole-4-carbaldehyde

Table 3: Spectral datas of AV2

Compound Code	IR	Streching	NMR	Interference	Mass
AV2	2922	Aromatic –CH stretching	7.5-7.9 – Multiplet	Ar-H	M^+ peak = 369.21
	2853	Aliphatic –CH- stretching	2.58 - Singlet	-CH_3	
	1583	Aromatic C=C stretching	8.98 - Singlet	-CHO	
	926	Aromatic C-C- stretching	6.87- Singlet	-N-CH-	
	1323	-C-N- stretching			
	1120	-N-N=C stretching			Base peak = 91.02
	1679	C=O stretching			
	755	-C-Br stretching			

Compound: AV3



1-(4-bromobenzoyl)-3-(4-bromo phenyl)-1H-pyrazole-4-carbaldehyde

Table 4: Spectral datas of AV3

Compound Code	IR	Streching	NMR	Interference	Mass
AV3	2923	Aromatic –CH stretching	7.5-7.9 – Multiplet	Ar-H	M^+ Peak: 434.08,
	2853	Aliphatic –CH stretching	8.98 - Singlet	-CHO	
	1585	Aromatic C=C stretching	6.87- Singlet	-N-CH	
	927	Aromatic C-C stretching			
	1322	-C-N- stretching			
	1120	-N-N=C stretching			Base Peak = 89.15
	1678	C=O stretching			
	755	-C-Br stretching			

3.3. Antibacterial Activity by Well Diffusion Method

Synthesized compounds have been evaluated for antibacterial activity by standard method against *Bacillus subtilis* (gram +ve) and *Pseudomonas aeuroginosa* (gram-ve).

All the tested compounds have been shown to exhibit significant antibacterial activity.

The results are presented in Table No: 5

Table 5: Antibacterial Activity of synthesized Compounds

Compound	Mean Zone Of Inhibition In mm					
	B.Subtilis (Gram +ve)			P.Aeuroginosa (Gram-ve)		
	100 µg/ml	250 µg/ml	500 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml
AV1	24	26	28	09	11	13
AV2	21	22	24	07	08	11
AV3	17	20	19	08	09	10
Control (DMSO)	-	-	-	-	-	-
Amoxycillin (50 µg/ml) (STD)	14			19		

3.4 MIC Determination

MIC of extract against gram positive organism such as *B.subtilis* and gram negative organism such as *P.aeuroginosa* were compared with the standard.

Table 6: MIC Data of synthesized compounds

Organism	Compound Code	100µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	3.125 µg/ml
B.S	AV1	S	S	S	S	R
	AV2	S	S	S	R	R
	AV3	S	S	S	R	R
	Std	S	S	S	S	S
P.A.	AV1	S	S	R	R	R
	AV2	S	R	R	R	R
	AV3	S	R	R	R	R
	Std	S	S	S	S	S

R= Resistant S= Sensitive

4. Conclusions

1-(4-bromobenzoyl)-3-(4-substitutedphenyl)-1H-pyrazole-4-carbaldehyde derivatives were prepared on the basis of extensive literature survey. All the synthesized compounds were screened for anti-bacterial activity by agar well diffusion method. The compounds exhibited moderate to good antibacterial activity against both Gram positive and Gram negative bacteria compared to the standard drug Amoxycillin.

5. Acknowledgments

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6. References

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