AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Screening of Antibacterial Activity of Novel Pyrazole Derivatives

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ABSTRACT

A new series of novel derivatives of pyrazole were synthesized. These derivatives were identified on the basis of melting point range, R_f values, IR and ^{1}H NMR spectral analysis. The derivatives were screened for antibacterial activity. All derivatives exhibited significant to moderate antibacterial activity.

Keywords: Antibacterial Activity, R_f values

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Received 01 October 2014, Accepted 07 October 2014

Please cite this article in press as: Verma AK *et al*., Screening of Antibacterial Activity of Novel Pyrazole Derivatives. American Journal of PharmTech Research 2014.

INTRODUCTION

Nitrogen containing heterocyclic compounds are synthetically the challenging models for a number of therapeutically significant products. Azoles occupy a domain of interest in natural and synthetic chemistry. Diazoles are the central building blocks for synthesizing compound libraries in pharmaceutical and agrochemical industries. One such class of compounds includes Pyrazole. Pyrazole refers to the simple doubly unsaturated compound containing two nitrogen (in neighbouring position) and three carbon atoms in the ring. The pyrazole nucleus is common in a number of biologically active molecules exhibiting antibacterial [1-2], antitubercular [3], anti depressant [4], anti-inflammatory [5-6], analgesic [7], anticancer [8-9], antioxidant [10-11] etc. activities**.** Research in this area is still unexplored, therefore the present study is directed towards the synthesis of novel derivatives of pyrazole with good yield and enhance antibacterial activity.

MATERIALS AND METHODS

All the chemicals procured from CHEMCO Labs, NICE chemicals. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Shimadzu FTIR system in KBr pellets and noted the absorption levels (cm^{-1}) were listed. ^1H NMR spectra were run on Bruker DPX 400 FTNMR in DMSO-d_6 as solvent and TMS as an internal standard. The Mass spectra were recorded on JEOL JMS600H mass spectrometer.

Step1: Synthesis of Ethyl-4-chlorobenzoate

P-chloro benzoic acid (30g) in ethanol was added with 150ml conc. sulphuric acid at 0.5 ^0C over a period of 30 min and refluxed for 2 hrs on a water bath. The reaction mixture was poured in to ice-cold water. The solid thus obtained was filtered, washed and dried. The dried product was recrystallised from ethanol to white needle shaped crystals.Yield:95.5% w/w.

Step2: Synthesis of 4-chloro benzohydrazide

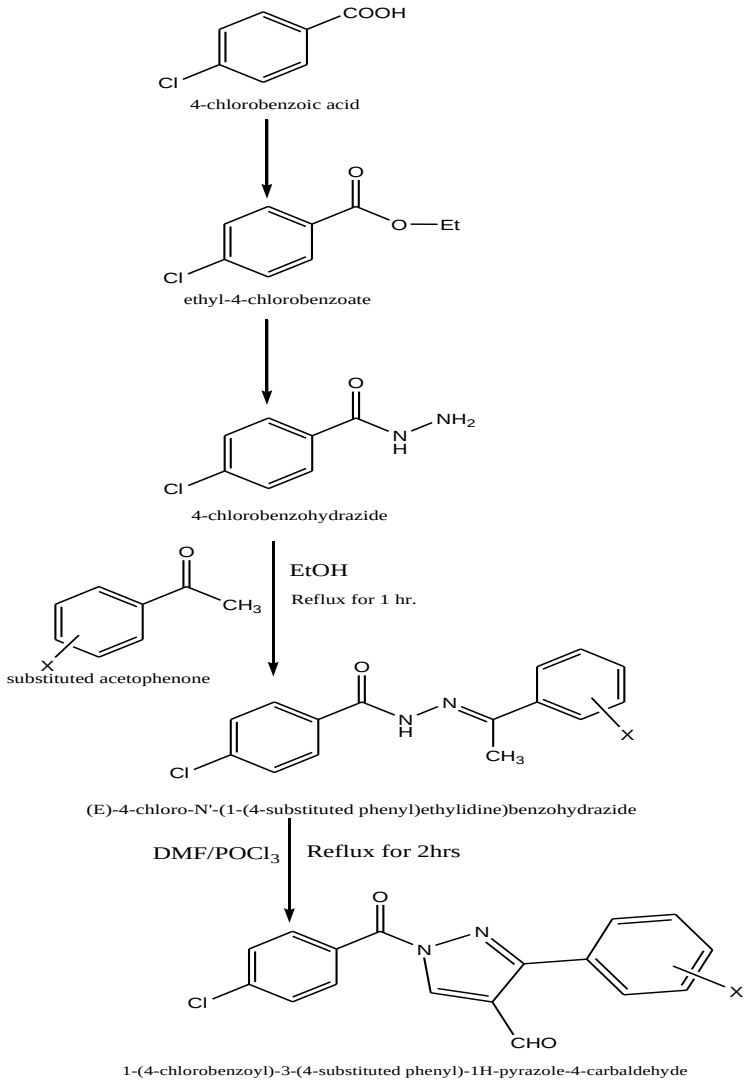
The mixture of 0.167 mol (29g) of substituted esters and 0.167 mol(5g)of hydrazine was warmed with 60ml of ethanol and few drops of glacial acetic acid. The reaction mixture was cooled and filtered. The solid thus obtained was washed with dil. HCl followed by about 12ml of cold rectified spirit. The dried product was recrystallised from ethanol to white needle shaped crystals of pure 4-chloro benzohydrazide.Yield:93% w/w.

Step3: Synthesis of (E)-4-chloro-N'-(1-(4-substituted phenyl)ethylidene) benzohydrazide

0.01mol of substituted acetophenone was added to the mixture containing 0.01mol of 4-chlorobenzohydrazide in 30ml of ethanol and few drops of glacial acetic acid. The reaction mixture was refluxed for 1 hr and then cooled in ice-bath. The product separated on cooling was filtered, dried and recrystallised from ethanol to white needle like crystals. (PZ1-PZ5)

Step 4: Synthesis of 1-(4-chlorobenzoyl)-3-(4 substituted phenyl)-1H-pyrazole-4-carbaldehyde

Cyclisation: The substituted hydrazone (0.005mol) was added in to the mixture of Vilsmeier-Haack (DMF&$POCl_3$) reagent, prepared by drop wise 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 2 hrs, then poured in to ice-cold water and neutralized using an excess of sodium bicarbonate solution. The product was washed with water and recrystallised from ethanol. (PZ1-PZ5).



X=p-methoxy,p-methyl,p-chloro,p-fluoro,p-bromo

Antibacterial activity [12]

Antibacterial activity of the synthesized derivatives was screened using the disc diffusion method against selected pathogens such as *Staphylococcus aureus, Escherichia coli,* The derivatives were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Nutrient agar (anti bacterial activity) was prepared and sterilized by an autoclave (121º C and 15 Ibs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45 ^{0}C Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized derivatives at a concentration of 500µg/ml, 300µg/ml and 150 µg/ml (*E.coli*) and 150 µg/ml, 100 µg/ml and 50 µg/ml(*S. aureus.)* were placed in the organism impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of derivatives at room temperature. Antibiotic discs of Ertapenam-10 mcg/disc, Netilmycin-30 mcg/disc and Streptomycin-100 µg/ml was used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 H at 37 ± 1. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.

RESULTS AND DISCUSSION

The melting points of all synthesized derivatives were found in open capillary tubes and readings were uncorrected. The structures of the synthesized derivatives were supported by physical data (Table 1) and following spectral analysis

Table 1: Physical data of the derivatives

Compound Code	R	MW(D)	MP(^{0}C)	Rf	Solvent system
PZ1	4-OCH3	342.783	201-203	0.67	Choloroform:Methanol (9:1)
PZ2	4-CH3	326.784	210-212	0.72	Choloroform:Methanol (9:1)
PZ3	4-Cl	347.202	192-194	0.60	Choloroform:Methanol (9:1)
PZ4	4-F	330.747	205-208	0.79	Choloroform:Methanol (9:1)
PZ5	4-Br	391.653	189-192	0.64	Choloroform:Methanol (9:1)

Only three compounds i.e. PZ1, PZ2 and PZ3 were taken for spectral studies. The results showed the presence of compounds which were predicted in the synthetic scheme.

1-(4-chlorobenzoyl)-3-(4-methoxy phenyl)-1H-pyrazole-4-carbaldehyde (PZ1)

IR (υ cm^{-1}): 3093(C-H, Ar-H),1087(C-Cl),1415(C=C),1712(C=O),1288(C-N)1174 (NN=C), 2665(C-H,aliphatic),808(C-C)1236(C-O-C),^{1}HNMR(DMSO-*d6*)δ:9.879(1H,S,-CHO),6.87(1H, S, -CH),3.34(3H,S,-OCH3),7.5-7.9(8H,M,-Ar), LC-MS: *m/z* 342.727(M$^+$).

1-(4-chlorobenzoyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde (PZ2)

IR (υ cm^{-1}): 3068(C-H, Ar-H), 1091(C-Cl),1425(C=C),1699(C=O),1282(C-N)1176(NN= C),

2954(C-H,aliphatic),819(C-C),^{1}HNMR(DMSO-*d6*)δ:8.975(1H,S,-CHO),6.79(1H,S,-CH),2.50 (3H,S,-CH3),7.5-7.9(8H,M,-Ar), LC-MS: *m/z* 326.516 (M^{+}).

1-(4-chlorobenzoyl)-3-(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde (PZ3)

IR (υ cm^{-1}): 3051(C-H, Ar-H)1091(C-Cl),1490(C=C),1668(C=O),1294(C-N)1128(NN= C), 2980(C-H,aliphatic),852(C-C),^{1}HNMR(DMSO-*d6*)δ:9.824(1H,S,-CHO),7.3-7.7(8H,M,-Ar), 6.176(1H,S,-CH), LC-MS: *m/z* 347.202(M^{+}).

Synthesized derivatives have been evaluated for antibacterial activity by standard method against *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve). Mean zone of inhibition of the derivatives were compared with different concentration of standard drugs. like Ertapenam (10 mcg/disk), Netilmicin (30 mcg/disk) and Streptomycin (100 mcg/ml) and DMSO as the control. All the tested derivatives have been shown to exhibit significant antibacterial activity. The results were presented in Table 2. Zone of inhibition of the derivatives against E.coli is given in figure.

Table 2: Antibacterial activity of derivatives

Compound	Mean Zone Of Inhibition In mm					
	S.Aureus(Gram+Ve)			E.Coli(Gram-Ve)		
	50	100	150	150	300	500
PZ1	26	29	34	25	26	29
PZ2	28	31	32	27	27	30
PZ3	29	30	33	28	29	32
PZ4	25	28	32	26	27	29
PZ5	27	29	31	29	29	32
Ertapenam(10 mcg/disc)	43			27		
Netilmicin(30 mcg/disc)	29			21		
Streptomycin 100 µg/ml	24			23		
DMSO	-			-		

'-' indicates no zone of inhibition

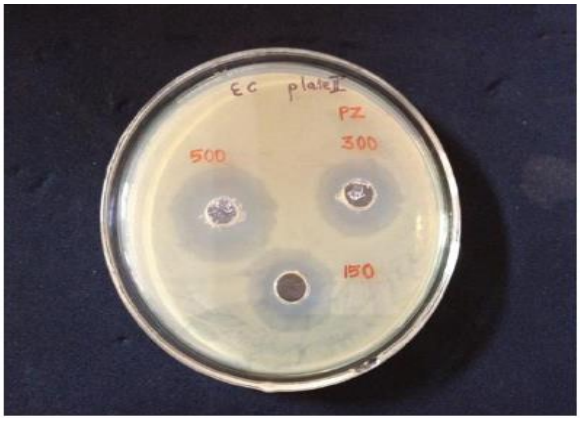


Figure 1: Zone of inhibition of the derivatives against E.coli

CONCLUSION

The research work was oriented towards the finding of novel derivatives of pyrazole with enhance antibacterial activity. The different derivatives were synthesized. The synthesized derivatives showed very good antibacterial activities against previously reported derivatives of pyrazole.

ACKNOWLEDGEMENT

The authors are thankful to Department of Chemistry, Magadh University, Bodh Gaya, Gaya, Bihar, India and Department of Pharmaceutical Chemistry, Spectrum Institute of Pharmaceutical Sciences and Research, Greater Noida, India for providing research facilities and encouragement and to our friends those helped us to complete this research.

REFERENCES

1. Sachchar SP, Singh AK, Synthesis and antibacterial activity of some novel pyrazole derivatives, Int. J. Comp. Socio., 1985; 12(3): 142-146.
2. Nada MA, Hamdi MH, Ahmed SM, Omar AM, Synthesis and antimicrobial evaluation of some new pyrazole, pyrazoline and chromeno [3,4-c] pyrazole derivatives, J. Bio.Chem., 2009; 20: 975-987.
3. Pattan SR, Synthesis and evaluation of some novel substituted 1,3,4-oxadiazole and pyrazole derivatives for antitubercular activity, Ind. J. Chem, 2009; 48(3): 1453-1456.
4. Mathew B, Suresh J, Anbazhagan S. Development of novel (1-H) benzimidazole bearing pyrimidine-torsine based MAO-A inhibitors: Synthesis, docking studies and anti depressant activity, J. Amer. Chem. Soc., 2012; 2(3): 1-8.
5. Kumar AS, Ilango K, Manikandan RS, Ramalakshmi N, Synthesis and anti-inflammatory activity of some novel pyrazole derivatives of gallic acid, Eur. J. Chem., 2009; 6(3): 123-128.
6. Sahu SK, Banerjee M, Samantray A, Behera C, Azam MA, Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel pyrazoline derivatives, Trop. J. Pharm. Res., 2008; 7(4): 961-968.
7. Panneer ST, Saravanan G, Prakash CP, Kumar PD, Microwave assisted synthesis, characterisation and biological activity of novel pyrazole derivatives, Afri. J. Chem., 2011; 1(2): 126-129.
8. Mohareb RM, El-Sayed NN, Adbelaziz MA, Uses of cyanoacetyl hydrazine heterocyclic synthesis: Novel synthesis of pyrazole derivatives with antitumour activity, Molecules, 2012; 17(2): 8449-8463.

9. El-Sayed WA, Flefel EM, Morsy MH, Anticancer and antimicrobial activities of some synthesized pyrazole and triazole derivatives, Der. Pharm. Chem,2012; 4(3): 23-32.
10. Bole SB, Nargund LV, Nargund R, Devaraju KS, Vedamurthy AB, Synthesis of substituted fluoro pyrazoles for antibacterial and anti-oxidant activity, Int. J. Pharm. Pharma. Sci., 2011; 3(3): 161-166.
11. Tarun S, Mithilesh, Raju S, Pooja C, Saraf, SK, Syntheses and antioxidant screening of pyrazole-4-carboxaldehyde derivatives, Int. J. Res. Pharm. Sci., 2012; 2(3): 81-96.
12. Sooley HW, Vandemark PY, A laboratory manual of microbiology, 4th ed., India: Teraporewala Sons and Co. Pvt. Ltd; 1995.