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COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITIES OF SOME BIS-PYRAZOLES AND BIS -PYRAZOLINES

Dr. Jyoti Shegokar

Department of Chemistry,

College of Engineering and Technology, Akola,Maharashtra,India.

E-mail : jyoti2511shegokar@gmail.com

1) Abstract :

A Series of substituted heterocycles, 1,1–{bis-[3-hydroxy-4-1-phenyl-5$^{|}$(3$^{|}$,4$^{|}$-disubstitutedphenyl)-pyrazol-3-yl]-oxyphenyl}-methanes (VIII)$_{(a-d)}$. and 1,1 – {bis-[3-hydroxy-4-1-phenyl-5$^{|}$-(3$^{|}$,4$^{|}$-disubstituted phenyl)-pyrozolin-3-yl]-oxyphenyl}-methanes–(V)have been synthesized by condensation of 1,1-{bis-[3-hydroxy-4-3-(3$^{|}$,4$^{|}$-disubstitutedphenyl)–propane-2-1,3-dione]-oxyphenyl}-methanes- (VIB) and 1,1-{bis-[3-hydroxy-4-3-(3$^{|}$,4$^{|}$-disubstituted phenyl)–prop-2-ene-1-one]-oxyphenyl}- methane (II) with phenyl hydrazine hydrochloride in pyridine medium respectively. The compounds were characterized by IR and ^{1}H NMR data. All the compounds are evaluated for their antibacterial and antifungal activity. Comparative study of these prepared bis-pyrazoles and bis-pyrazolines have been carried out in this paper.

Keywords : Bis-pyrazoles, Bis-pyrazolines,antimicrobial activity.

Introduction :

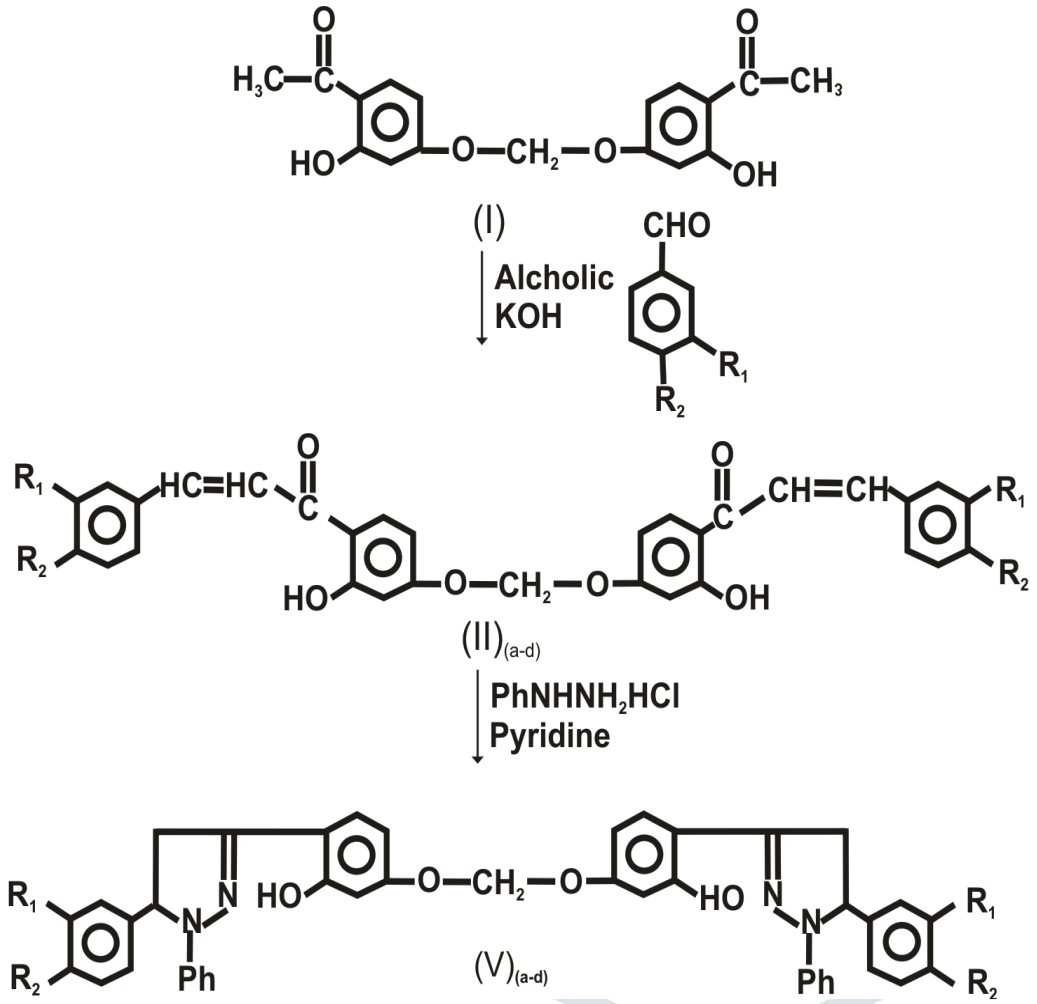
Heterocyclic compounds are fundamentally important to biological processes and are widely sprayed as a natural products. They are predominantly used as a pharmaceuticals, as agrochemicals and veterinary products. They also find applications as antioxidants antibacterial,antifungal activities etc.

Chalcones are well known intermediated for synthesizing various heterocyclic compounds,chalcones are of considerable interests for both synthetic, organic and medicinal properties. Pyrazolines are conveniently synthesized by the action of hydrazine or phenyl hydrazine hydrochloride on chalcone[1] pyrazoline[2] is a class of organic compounds consisting of unsaturated five membered ring containing adjacent hetero atom. And pyrozoles[3,4] are commonly synthesized by the action of hydrazine or substituted hydrazine on 1, 3- dicarbonyl compounds.

Scheme :

SCHEME-1

Synthesis of 1,1-{Bis-[3-hydroxy-(4-phenyl-5'(3',4'-disubstituted phenyl pyrazolin -3-yl)-oxyphenyl}-methane $\mathbf{V}_{(a\text{-}d)}$



Where (V)a →1,1 – {bis-[3-hydroxy-4-1-phenyl-5'-(3',nitro phenyl)-pyrozolin-3-yl]-oxyphenyl}-methane

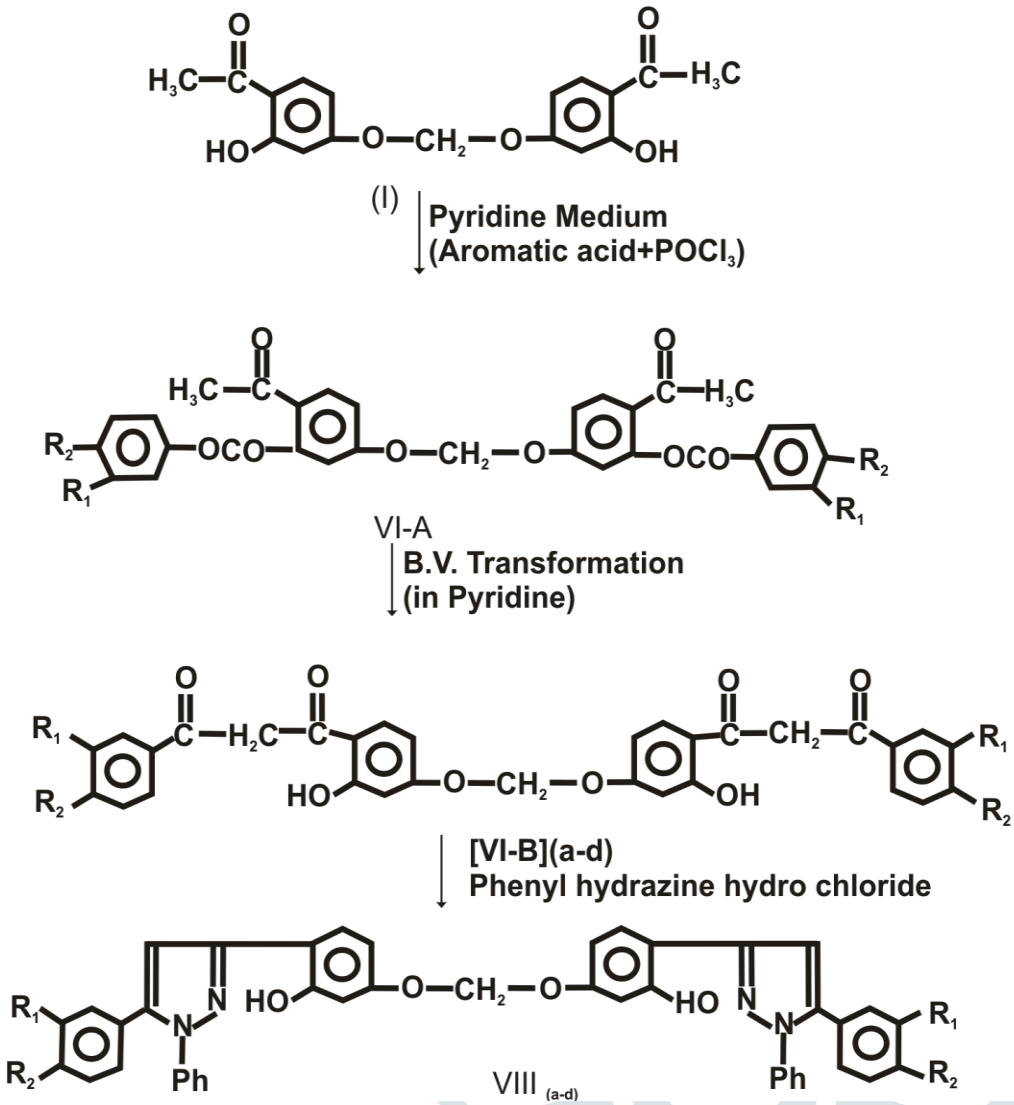
(V)b→1,1 – {bis-[3-hydroxy-4-1-phenyl-5'-(3',hydroxy phenyl)-pyrozolin-3-yl]-oxyphenyl}-methane

(V)c→1,1 – {bis-[3-hydroxy-4-1-phenyl-5'-(4' -nitrophenyl)-pyrozolin-3-yl]-oxyphenyl}-methane

(V)d→1,1 – {bis-[3-hydroxy-4-1-phenyl-5'-(3',4' -dimethoxy phenyl)-pyrozolin-3-yl]-oxyphenyl}-methane

SCHEME-2

Synthesis of 1,1-{Bis-[3-hydroxy-(4-phenyl-5'(3',4'-disubstituted phenyl pyrazol -3-yl)-oxyphenyl}-methane **VIII**$_{(a-d)}$



Where (VIII)a → 1,1–{bis-[3-hydroxy-4-1-phenyl-5'(3', -nitrophenyl)-pyrazol-3-yl]-oxyphenyl}-methane

(VIII)b → 1,1–{bis-[3-hydroxy-4-1-phenyl-5'(3', -hydroxyphenyl)-pyrazol-3-yl]-oxyphenyl}-methane

(VIII)c→ 1,1–{bis-[3-hydroxy-4-1-phenyl-5'(4', -nitrophenyl)-pyrazol-3-yl]-oxyphenyl}-methane

(VIII)d→ 1,1–{bis-[3-hydroxy-4-1-phenyl-5'(3',4'-dimethoxyphenyl)-pyrazol-3-yl]-oxyphenyl}-methane

Result and Discussion :

Present work deals with the comparative study of antimicrobial activities of synthesized **, 1,1–{bis-[3-hydroxy-4-1-phenyl-5^{I}(3^{I},4^{I}-disubstitutedphenyl)-pyrazol-3-yl]-oxyphenyl}-methanes (VIII)$_{(a-d)}$. and 1,1 –{bis-[3-hydroxy-4-1-phenyl-5^{I}-(3^{I},4^{I}-disubstituted phenyl)-pyrozolin-3-yl]-oxyphenyl}-methanes–(V)**. Melting points are uncorrected .IR spectra were recorded on SHIMADZU FTIR-IRAffinity-1 (4000-500cm^{-1}) , ^{1}HNMR spectra were recorded on Brucker AC 300 NMR spectrometer at 300 MH$_z$ in Acetone. Purity of compounds was checked by TLC.

The compounds **VIII** $_{(a-d)}$ and **V** $_{(a-d)}$ were tested for antimicrobial activity against bacterias like Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pseudomoniae , Escherichia coli, Salmonella abony and fungi like Candida albucans , Saccharomycae cerevisia using agar disc-diffusion method at a 25□g/ml. in DMSO as a solvent. After 24 hour of inhibition at 37^{0}C the zone of inhibition were measured in mm. The values were recorded in the table 1 and 2.

EXPERIMENTAL :

I) General Procedure for Preparation of bis-chalcone :-

1,1-{bis-[3-hydroxy-4-acetyl] oxyphenyl}- methane (I) (0.01 mole) and aromatic aldehyde (0.02 mole) dissolved in 40 ml ethanol. The reaction mixture was warmed up to 50^{0}C ,KOH (0.04 mole) was added to reaction mixture with constant stirring. The reaction mixture was kept overnight. The crude product obtained was decomposed by 50% AcOH.

II) Preparation of bis-pyrazolines : -

A mixture of 1,1-{bis-[3-hydroxy-4-3-(3□,4□-disubstituted phenyl)–prop-2-ene-1-one]-oxyphenyl}-methane (II) (0.0075 mole) and phenyl hydrazine hydrochloride (0.005 mole) was refluxed in pyridine (in 40 ml.) for four hours. The reaction mixture was cooled and diluted with water a yellow crude product obtained was crystallized from ethanol.

III) Preparation of Bis-diketone (VIb)

Step-I

1,1-{bis-[3-hydroxy-4-acetyl] oxyphenyl}- methane (I) (0.0025mole) and substituted aromatic acid (0.005mole) was dissolved in a dry pyridine (10ml). The reaction mixture was treated with $POCl_3$ and temperature was maintained below 40^{0}C. The reaction mixture was slowly thickened. After 5 hours, it was treated with cold dil HCl (i.e.1:1) The product obtained was washed with water and then by sodium carbonate (10%) to remove any un reacted organic acid. The product obtained was washed with dil sodium hydroxide (1%) to remove unreacted ketone. The product was finally washed with water and crystallized with ethanol, Product **(VIa)** named Bis-(1-acetyl-2-aroyloxy-4-oxyphenyl) –methane.

Step – II

Compound Bis-(1-acetyl-2-aroyloxy-4-oxyphenyl) –methane. **(VIa)** (1.30gm) was dissolved in pyridine (anhydrous) 5 ml at about 50^{0}C in pulverized KOH (1gm) was added with constant stirring.

The reaction mixture was kept stand by for overnight. And it was decomposed by ice cold (25%) HCl. The crude product obtained was washed by $NaHCO_3$(10%). Solution to remove any mineral acid and then crystallized by ethanol. i.e. 1,1-{bis-[3-hydroxy-4-3-(3^{I},4^{I}-disubstitutedphenyl)–propane-2-1,3-dione]-oxyphenyl}-methanes- (VIB).

IV) Preparation of Bis-pyrazoles – VIII $_{(a-d)}$

Compound VIB (i.e. bis-diketone) (0.0025 mole) and $PhNHNH_2$ HCl (0.01mole) were refluxed in 10 ml of pyridine for about 3 hours.

After cooling, the reaction mixture was diluted with 50% water and washed with (50%) acetic acid solution followed by water and the product was crystallized from ethanol.

Spectral Interpretation:

1) **Compound – VIII (a-d)-IR-Spectra.**

Sr. No.	Frequency	Inlensity	Correlation
1	3321.12	s	Ar-OH-Stretcting
2	2918.30	s	Methyl-C-H- Stretcting
3	1631.78	d	C=N- Stretcting
4	1427.32	s	C=C- Stretcting
5	1363.67	s	C-N- Stretcting
6	1012.63	s	(O-C) Stretcting

2) **^{1}H NMR-spectra of compound VIII (a-d)**

^{1}HNMR analysis showed signals due to aromatic protons, pyrazole protons and (-CH_2-) methylene protons.

3) IR-Spectra-compound V (a-d)

Sr. No.	Frequency	Inlensity	Correlation
1	3331.07	s	Ar-OH
2	2918.30	s	Methyl-(C-H)- Stretcting
3	1602.85	s	C=N- Stretcting
4	1502.55	s	C=C- Stretcting (phenolic)
5	1251.80	s	(C-O)
6	1008.77	s	-(C-O) Stretcting
7.	1417.68	s	C-N- Stretcting

4. NMR-Spectra of compound V (a-d)

The ^{1}HNMR spectrum of the product distinctly showed signals due to, aromatic protons, methylene protons and pyrazoline protons. (i.e.-CH_2 and-CH).

Test procedure:

At first media was prepared by dissolving weighed ingredients and was sterilized at 121^0 c temperature and 15 Ibs/ inch^2 pressure for 15 minutes. After sterilization it was cooled about 40^0 c and poured in to sterile petridishes and allowed to solidify. The media plants were seeded with 24 hours old active nutrient broth culture (1ml/plate) of the test organism in order to obtained lawn culture.

The stainless steel cork borer of 8mm diameter was used to make the cavities called wells.

Each compound was taken at a concentration 0.1 mg/1ml using acetone as a solvent.

The portion of test compound dissolved in a solvent was added in to the bore up to filling it. The drug solution(prepared compounds with solvent) was allowed to dry for about 2 to 3 hours.

The zone of inhibition observed around the wells after respective incubation was measured in millimeter by using 'antibiotic zone reader the results were cited in tables..

The medium used for antibacterial study was Hi media Antibiotic medium No.11.

Antibiotic medium No.11 :-

(Composition -	**Grams /litre)**
1. Peptone	6.000
2. Pancreatic digest of casein	4.000
3. Yeast Extract	3.000
4. Beef Extract	1.500
5. Dextrose	1.000
6. Agar	15.000
P^H	8.3 to 1

The medium used for antifungal activities were Hi-media Sabouraud Dextrose Agar.

Sabouraud Dextrose Agar :-

Composition	**Grams / litre**
1. Dextrose (Glucose)	40.000
2. Mycological, peptone	10.000
3. Agar	15.000
P^H	5.6 to 0.2

The compounds were taken at a concentration of 1ug/ml using dimethyl sulphoxide (DMSO) as solvent.

The plants were incubated at 37^0C for 24 hours and 30^0C for 48 hours for antibacterial and antifungal activities respectively.. The results were as follows –

Table a) : An Antimicrobial activities of 1, 1-{bis-[3-hydroxy-4-phenyl-5'-(3',4'-disubstituted phenyl)–pyrazol-3-yl]-oxyphenyl}- methane (VIII)(a-d)

Organism - Compounds|	E.coli	S.aureus	P. aeruginose	S. epidermisdis	S. abony	C. albicans	S. cerevisia
VIIIa	++	++	++	++++	+++	+++	++
VIIIb	--	++	++	+++	+++	++++	+++
VIIIc	+++	++	++	+++	++	++++	---
VIIId	++	--	+++	+++	++	+++	+++

NB:- ++++ : Strongly active (above 20 mm)
+++ : Moderately active (15 mm to 20 mm)
++ : Weakly active (8 to 14mm)
- - : inactive (below 8 mm)

The graphical representation of above is shown below:

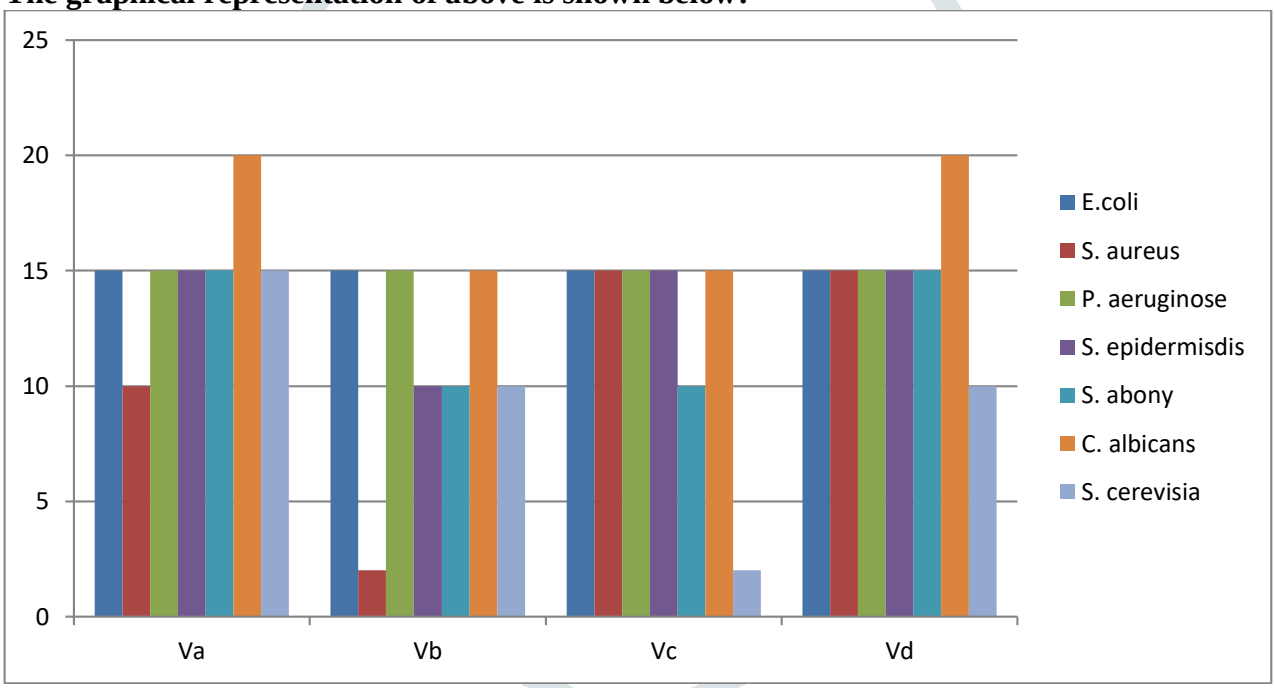
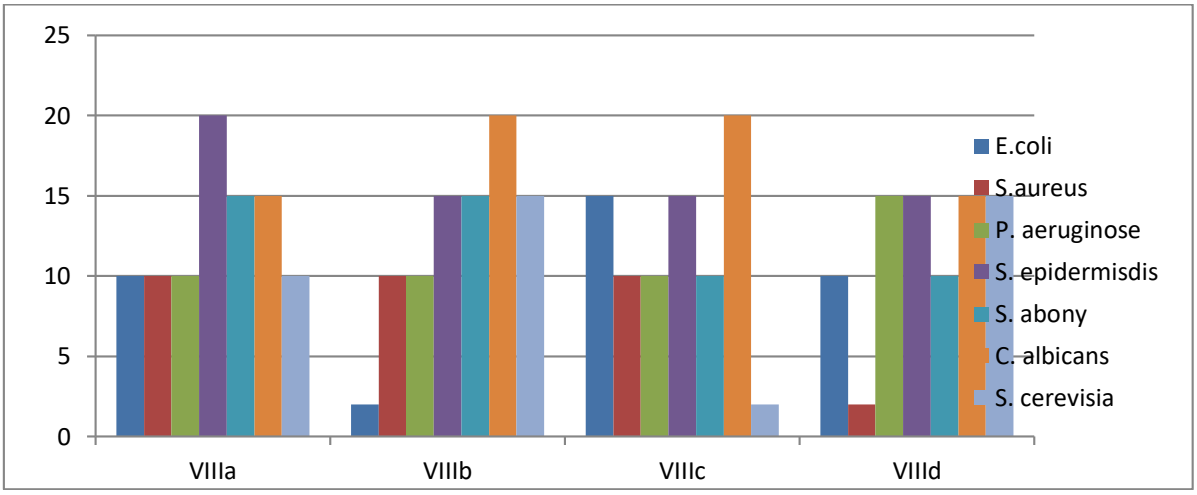


Table 2 b) : Antimicrobial activities of compound V(a-d)

Organism – Compounds |	E.coli	S. aureus	P. aeruginose	S. epidermisdis	S. abony	C. albicans	S. cerevisia
Va	+++	++	+++	+++	+++	++++	+++
Vb	+++	---	+++	++	++	+++	++
Vc	+++	+++	+++	+++	++	+++	---
Vd	+++	+++	+++	+++	+++	++++	++

NB:- ++++ : Strongly active (above 20 mm)
+++ : Moderately achive (15 mm to 20 mm)
++ : Weakly active (8 to 14mm)
- - : Inactive (below 8 mm)
Bore Size – 8mm

The graphical representation of above is shown below:



Result and Discussion-

In Pyrazoles, all the compounds are almost strongly active towards Candida albicans while they are weakly active towards E.coli, S. aerureus and P. aeruginose. And showed moderate activities towards said organisms while VIIIb i.e. m-nitro,VIIIc ,p-nitro and VIIId dimithoxy, pyrazoles are inactive towards E.coli, S.aereous and S.cerevisia

All prepared pyrazoline compounds are moderately active towards E. coli and P. aeruginose while shows strong activity towards C. albicans. S.cerevisia microbe is more active towards prepared chemicals.

Vb and Vd are inactive towards S.aureus and S.cerevisia.

Overall the Both heterocyclic compounds are moderately active towards said organisms.

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