International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Biological Study of Synthesised E/Z-4, 5-Dihydro Spiro [3-Phenyl-5-Substituted Phenyl Isoxazole-4, 4' (2',4'-Dihydro-5'-Methyl-2'-Phenyl/ Phenyl Methyl-3'h-Pyrazol-3'-Ones)]

Vipin Kumar Singh[1], Vijay Pratap Singh[2], Amrendra Kumar Singh[3]

[1]Department of Chemistry, Tilak Dhari Post Graduate College, Jaunpur, U.P.-222002, India
Email: *vipinksingh24[at]gmail.com*

[2]Department of Chemistry, Faculty of Engineering & Technology, VBS Purvanchal University, Jaunpur, U.P.-222003, India
Email: *vpsingh.chem[at]gmail.com*

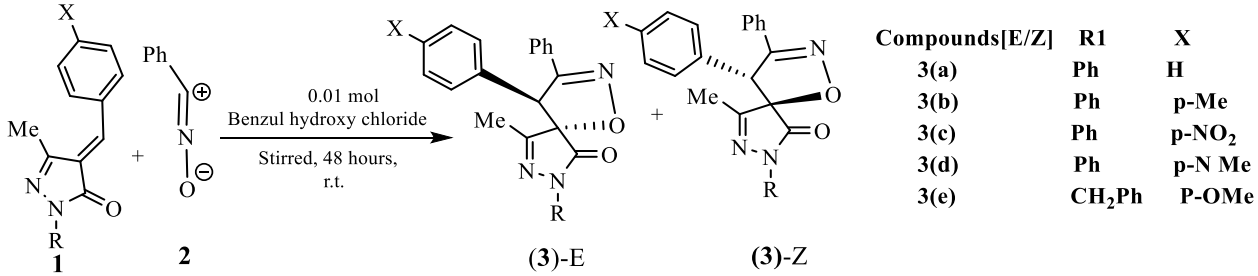
[3]Department of Chemistry, Faculty of Engineering & Technology, VBS Purvanchal University, Jaunpur, U.P.-222003, India
Email: *aks.vbspu[at]gmail.com*

Abstract: ***A study on biological activity of synthesized [E-(3a-e) and Z-(3a-e)] spiro isoxazole-pyrazolone derivatives. This examination by single spore isolation and disc diffusion method shows that all compounds have antimicrobial activity. The compounds 3d (E/Z) shows best antibacterial activity against Gram positive bacteria Staphylococcus aureus, Bacillus subtilis, Gram negative bacteria Pseudomonas aeruginosa, Escherichia coli and antifungal activity against Aspergillus niger.***

Keywords: Spiro isoxazole-pyrazolones, Antibacterial activity, Inhibition zones, Single spore isolation, Disk diffusion

1. Introduction

Pyrazoles are five membered most important heterocyclic molecules [1]. Spiro-Pyrazolone derivatives are heterocyclic, having broad spectrum of biological activities, antibacterial, anti-inflammatory, antiviral, antifungal, antitubercular, antitumor [2], antioxidant, Anticancer [3] and analgesic, with outstanding pharmacological [4]. Spiro-pyrazolone derivatives have been patented due to their antimicrobial activity [5, 6]. After recognizing the importance of to drugs, pyrazole and isoxazoline, we were interested in preparing the compounds containing both rings. The pyrazolone spiro-fused isoxazoline derivatives were prepared by using following scheme [7].



Compounds[E/Z]	R1	X
3(a)	Ph	H
3(b)	Ph	p-Me
3(c)	Ph	p-NO_2
3(d)	Ph	p-N Me
3(e)	CH_2Ph	P-OMe

Scheme 1: Synthesized Spirocyclic Pyrazolone derivatives [E/Z, 3(a-e)]

The objective of present study is investigation of biological activity of synthesized E/Z-4, 5-Di hydro spiro [3-phenyl-5-substituted phenyl isoxazole-4, 4' (2', 4,-dihydro-5-methyl-2'-phenyl/ phenyl methyl-3'H-pyrazole-3'-ones)] compounds. The presence of imino or azo group enhances its biological activity. There for we focused for study of biological effects of these molecules. We investigated the biological activities of all the synthesized title compounds for their antibacterial and antifungal activity against bacteria Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and fungi Aspergillus niger [8-13], isolated from hosts (seeds of Soybean, Maize, Gram seeds) directly [14, 15, 16].

2. Experimental

Characterization of synthesized products was done by suitable instruments. The melting points determination on a Buchi apparatus and were uncorrected. Microanalyses were carried out on Coleman C, H, and N-analyzers. IR spectra (Nujol) were recorded on Perkin-Eimer-720 and 257 spectrophotometers and PMR spectra ($CDCl_3$) on Varian A-60D and Jeol FX-90Q spectrometers using TMS as an internal standard for Compounds [E/Z, 3(a-e)].

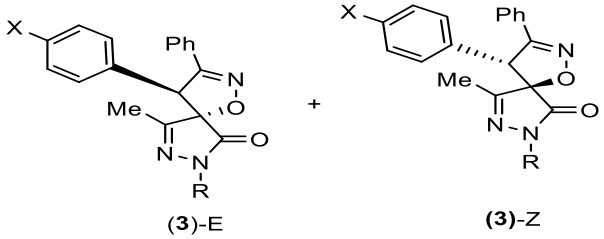
Compounds [E/Z, 3(a-e)] were evaluated in vitro using the Nutrient Agar and Sabouraud Dextrose Agar, by disc diffusion methods, DMF as solvent. Bacterial sub-culture in nutrient Agar, where as Fungal sub-culture in Sabouraud

dextrose Agar was carried out. Inoculums was prepared from the above bacterial culture, 4 to 5 colonies are transferred in 5 mL 0.9% saline with the help of wire loop, incubated at 35^0 C. When it exceeds turbidity of 0.5 Mac Farland standards, reduce the turbidity by adding same saline. Plates were dried and 1 mL of each standardized inoculums suspension was poured and uniformly spread. Amphotericine-B, Ciprofloxacin, and Amoxicillin, were used as standard compound as antibacterial and antifungal agents, DMF alone showed no inhibition zone. The plates were incubated at 35°C for 24 h. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth around the disks in mm.

3. Results and discussions

In present aim for biological study of synthesized compounds [E/Z, 3(a-e)], we have done evaluation of antibacterial, antifungal activity against Gram positive and Gram negative bacteria and fungi. We find that all are biologically active compounds. The compound **3d (E/Z)** is considerably more active against both bacteria and fungi. Most of the synthesized compounds bearing electron donating group at para position of phenyl group were found to be exhibiting more biological activities. Previous study also supported the findings. **We will continue this study with 3d (E/Z), for further modifications in structure and biological activity.**

Table 1: Synthesized compounds [E/Z, 3(a-e)]



(3)-E (3)-Z

Compounds	R	X
3(a)	Ph	H
3(b)	Ph	p-Me
3(c)	Ph	p-NO_2
3(d)	Ph	p-N Me
3(e)	CH_2Ph	P-OMe

4. Biological Activity

Since Spirocyclic pyrazolone and isoxazoline derivatives are reported well for their various biological activities. These have good antipyretic, antitubercular, antiarthritic, anti-inflammatory and antitumor activities. Looking their biological activities, we are highly interested for the study of biological activity of our synthesized compounds in special reference of their antimicrobial properties. The bactericidal and fungicidal activity of all [E/Z, 3(a-e)], synthesized Spirocyclic pyrazolones has been screened and data is given in Table-2.

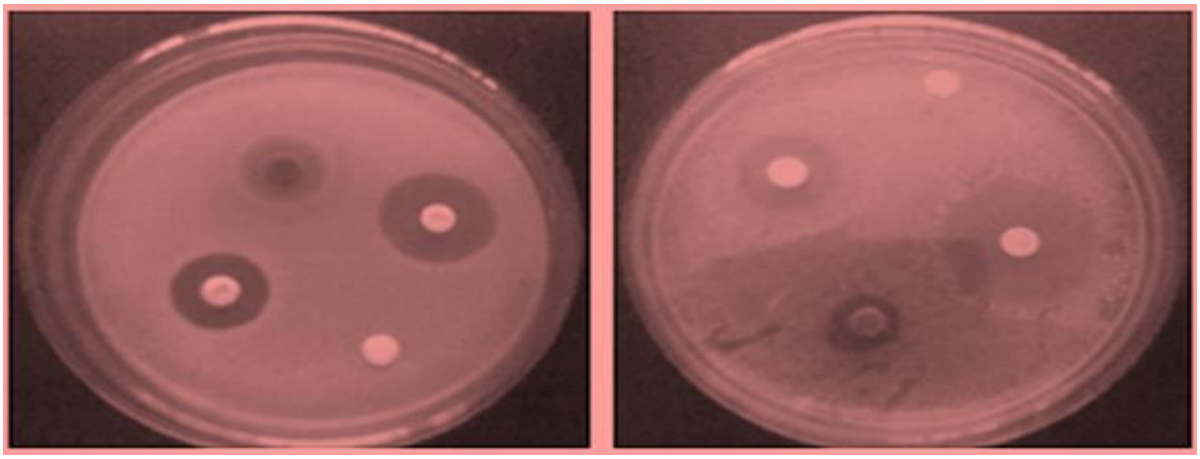


Figure 1: Antibacterial efficiency of **3d, (E/Z)** against Gram-positive Staphylococcus aureus and Bacillus subtilis (A and B) respectively

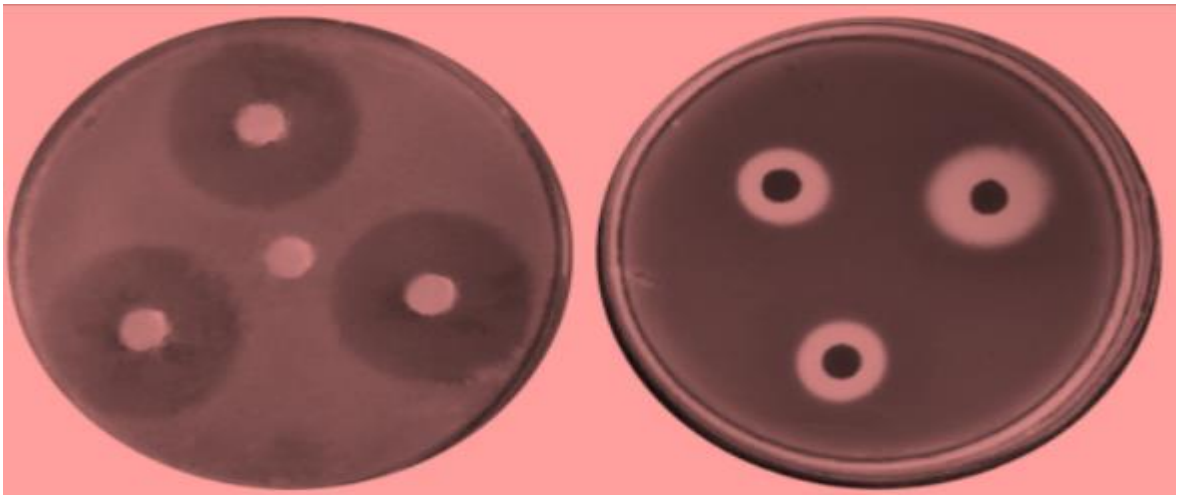


Figure 2: Antibacterial efficiency of **3d (E/Z)** against gram-negative bacteria Pseudomonas aeruginosa and Escherichia coli (C and D) respectively

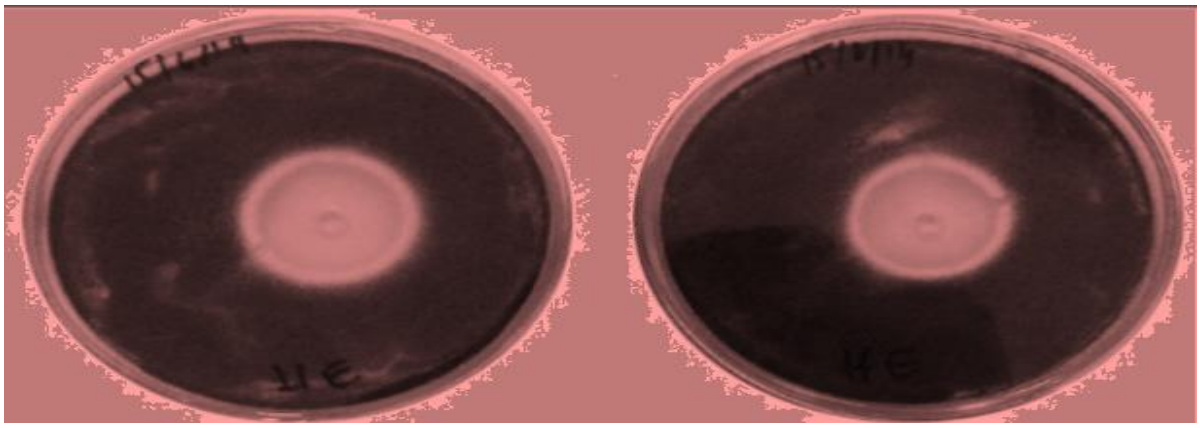


Figure 3: Antifungal efficiency of Spirocyclic pyrazolone 3d (E and Z) respectively against Aspergillus niger

Table 2: Data of Bacterial / antifungal activity for Spiro-cyclic Pyrazolones

S.N.	Spirocyclic pyrazolones	Gram (+) Bacteria S. aureus	B. Subtilis	Gram (-) Bacteria P. aeruginosa	E. coli	Fungi A. nigar
1	3a, E	20	15	27	14	16
2	3a, Z	13	25	19	12	12
3	3b, E	19	22	25	16	18
4	3b, Z	11	22	24	15	14
5	3c, E	21	14	28	14	16
6	3c, Z	14	26	25	17	13
7	3d, E	22	23	24	22	23
8	3d, Z	23	24	22	20	21
9	3e, E	20	15	27	14	13
10	3e, Z	12	22	13	15	12
K	Amphotericine-B	-	-	-	-	26.8
L	Ciprofloxacin	-	-	30.2	25.8	-
M	Amoxicillin	25.6	28.3	-	-	-

*Inhibition zone in mm of Spirocyclic Pyrazolones and reference drugs for bacteria/ fungi

4.1 Antibacterial activity

The evaluation of compounds [E/Z, 3(a-e)], shows that the molecule **3d, (E/Z)** shows best antibacterial/ antifungal activity against all selected micro-organisms, having inhibition zone nearest to their reference drugs, due to presence of p-NMe_2 group neighboring to methyl and phenyl group and N, O interaction. The compound **E isomers of 3(a-e)** are highly active against **S. aureus** having inhibition zone 20, 19, 21, 22, 20 mm near to that of Amoxicillin due to presence of ring-oxygen and phenyl groups methyl phenyl, nitro group in different plane. The **Z isomers of 3(a-e)** are highly active against **B. subtilis** having carbonyl oxygen hindered by phenyl group and p-nitro phenyl groups, where as few **E** isomers are also active **3b, E & 3d, E** having inhibition zone 22, 23. All compounds are active against **P. aeruginosa** with moderate to good activity **3a, Z & 3e, Z,** having very low inhibition zone 19, 13. All compounds have low activity against **E. coli,** only 3d(E/Z)have considerable activity having inhibition zone 22, 20 nearest to reference drug Ciprofloxacin with rang of 25.8.

4.2 Antifungal activity

The evaluation of compounds [E/Z, 3(a-e)], shows that the compounds **3d [E/Z]** are highly active against **A. niger** with inhibition zone 23, 25, near to reference drugs Amphotericine-B having that of 26.8 mm, due to presence of carbonyl oxygen between p-NMe_2, phenyl groups. Other compounds also show antifungal activity with moderate to low range.

5. Conclusions

Here we have done the evaluation of synthesized all [E/Z, 3(a-e)] compounds for their anti-bacterial properties against Gram-positive bacteria Staphylococcus aureus, Bacillus subtilis and Gram-negative bacteria Pseudomonas aeruginosa, Escherichia coli and antifungal properties against fungus Aspergillus niger by disc diffusion method. All are active against these micro-organisms. The compounds **3d (E/Z)** shows valuable antimicrobial activity. We will continue our study with this compound for further modifications.

Acknowledgement
We are highly thankful to VBS Purvanchal University Jaunpur for providing us financial support, and Deptt. of chemistry, Faculty of Engineering & Technology and Department of Chemistry, Tilak Dhari Post Graduate College, Jaunpur, for providing opportunity of lab.

References

[1] Arora P, Arora V, Lamba HS, Wadhwa D. 2012. Importance of Heterocyclic Chemistry: a Review. International Journal of Pharmaceutical Sciences and Research, 3(9) :2947–2954.
[2] Kendre BV, Landge MG, Jadhav WN, Bhusare SR. 2013. Synthesis and bioactivities of some new 1H-pyrazole derivatives containing an aryl sulfonate moiety. Chinese Chemical Letters, 24(4): 325–328. https://doi.org/10.1016/j.cclet.2013.02.016
[3] Rokade YB, Sayyed RZ. 2009. Naphthalene derivatives: A new range of antimicrobials with high therapeutic value. Rasayan Journal of Chemistry, 2(4): 972–980.
[4] Milad T, Haniyeh A, Hamideh N. 2014. Mild and efficient synthesis of 1-(6-chloroquinoxalin-2-yl)-2-[4-(trifluoromethyl)-2,6-dinitrophenyl] hydrazine derivatives by microwave irradiation. Chemical Research in Chinese Universities, 30(3) : 405–408. https://doi.org/10.1007/s40242-014-3417-3
[5] Azam F, Singh S, Khokhra SL, Prakash O. 2007. Synthesis of Schiff bases of naphtha[1,2-d]thiazol-2-amine and metal complexes of 2-(2'-hydroxy) benzylideneaminonaphthothiazole as potential antimicrobial agents. Journal of Zhejiang University, Science B, 8(6): 446–452. https://doi.org/10.1631/jzus.2007.B0446.
[6] Farag AM, Mayhoub AS, Barakat, SE, Bayomi AH. 2008b. Synthesis of new N-phenylpyrazole derivatives with potent antimicrobial activity. Bioorganic and Medicinal Chemistry, 16 (8): 4569–4578. https://doi.org/10.1016/j.bmc.2008.02.043
[7] Amrendra Kumar Singh, Vijay Pratap Singh Heterocyclic Letters Vol. 13| No.4|775-779|Aug-Oct|2023 ISSN : (print) 2231–3087 / (online) 2230-9632 CODEN: HLEEAI http://heteroletters.org
[8] Adams, M. Staphylococcus aureus and other pathogenic Gram-positive cocci. In Foodborne Pathogens; de Blackburn, C.W.,McClure, P.J., Eds.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Elsevier: Cambridge, UK, 2009;pp. 802–819. ISBN 9781845693626.
[9] Schaumburg, F.; Pauly, M.; Anoh, E.; Mossoun, A.; Wiersma, L.; Schubert, G.; Flammen, A.; Alabi, A.S.; Muyembe-Tamfum, J.-J.;Grobusch, M.P.; et al. Staphylococcus aureus complex from animals and humans in three remote African regions. Clin. Microbiol. Infect. **2015**, 21, 345.e1–345.e8.
[10] J. Errington and Lizah T van der Aart, Errington and Aart, Microbiology 2020;166:425–427 DOI 10.1099/mic.0.000922
[11] James A. Driscoll, Steven L. Brody and Marin H. Kollef Drugs 2007; 67 (3): 351-368 REVIEW ARTICLE 0012-6667/07/0003-0351/$49.95/0
[12] "coli". Oxford English, Dictionary *(Online ed.)*. Oxford University Press. (Subscription or participating institution membership required)
[13] Wells, J. C. (2000) Longman Pronunciation Dictionary. Harlow [England], Pearson Education Ltd.
[14] Tenaillon O, Skurnik D, Picard B, Denamur E (March 2010). "The population genetics of commensal Escherichia coli". Nature Reviews. Microbiology. 8 (3): 2007-17 doi: 10.1038/nrmicro2298. PMID20157339. S_2CID 5490303.
[15] *Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley. pp. 444–54.* ISBN 978-0-471-98880-9.
[16] Checinska A, Probst AJ, Vaishampayan P, White JR, Kumar D, Stepanov VG, Fox GE, Nilsson HR, Pierson DL, Perry J, Venkateswaran K. 2015. *Microbiome* 3:50. Crossref. PubMed.