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SYNTHESIS, SPECTRAL STUDY AND ANTIMICROBIAL ACTIVITY OF NEWLY CHALCONES, PYRAZOLINES MOLECULES BEARING IN PYRIDINES NUCLEUS.

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***Abstract*:** A new series of compounds, namely1-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl) methoxy) phenyl)-3-arylprop-2-en-1-ones (3a-3j) and 3-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-ones (4a-4j), were synthesized. The chemical structures of these compounds were confirmed by ^{1}H-NMR, IR, Mass spectral analysis. The compounds (3a-3j) and (4a-4j) have been evaluated their antimicrobial activity.

***Keywords*:** Chalcones, Pyrazolines, Anti-bacterial & Anti-fungal activity (Heterocyclic compounds).

I. INTRODUCTION :

Chalcone are a type of organic compounds that belongs to the flavonoid family[1]. They consist of two aromatic rings connected by a three-carbon α,β-unsaturated carbonyl system[2]. Chalcones have industrial importance and are also used in the medicinal field. Chalcones are used to synthesize Pyrazoline, Pyrimidine, Pyrazole, and Thiosemicarbazide derivatives because of highly reactive unsaturated pi-bond[3]. Existing data shows that nitrogen-containing heterocyclic compounds such as pyrazoline have medicinal importance because of their biological activities[4]. Pyrazolines are another class of important heterocyclic compounds containing a five-membered ring with two nitrogen atoms and three carbon atoms[5-6]. The combination of chalcone and pyrazoline moieties allows medicinal chemists to create hybrid molecules with a broad range of biological activities[7-8].

Chalcones and pyrazolines have been found to exhibit various biological properties, such as Antibacterial[9-10], Antifungal[11-12], Antitumor[13], Anti-inflammatory[14], Anthelmintic[15], Anticancer[16], Anti-HIV[17], Antihypertensive[18], Antihistaminic[19], Anti-oxidant[20], Anti-convulsant[21] and Analgesic[22] activities.

Our research work focuses on synthesized heterocyclic compounds derived from chalcones, which exhibit many biological activities and find various applications in industries. In our study, we have dedicated our efforts to synthesis, of pyrazoline derivatives (4a-4j) derived from chalcones (3a-3j). Subsequently, we evaluated the antimicrobial properties of these newly synthesized chalcones and pyrazolines derivatives and compared them with known standard drugs using the cup-plate method[23].

The cup-plate method is commonly used to assess the antimicrobial activity of compounds. This method depends on the diffusion of an antibiotic from a vertical cavity, through the agar layer containing microorganisms in a petri plate. In this method, prepare an agar plate, and a swap of pure bacterial culture is evenly spread on the agar plate. Then, the synthesized compounds are added to the agar plate containing microorganisms, now kept this petri plate for incubation for 24 to 30 hours to allow them to diffuse and come into contact with the microorganisms. After an incubation period, a clear area (Zone of inhibition) around the tested sample is observed and measured. This measured zone indicates the antimicrobial activity of the compounds.

II. RESEARCH METHODOLOGY :

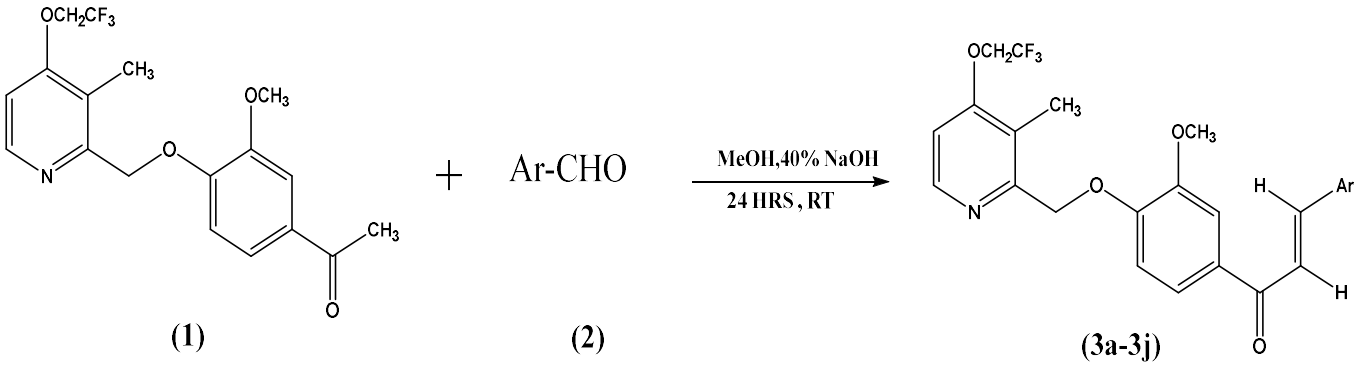
The synthesis process involved the use of analytical grade (AR) chemicals sourced from SRL and Phenar companies, which were employed without any additional purification. All reactions took place under specified conditions. The purity of the synthesized compounds was assessed using TLC with silica gel G (Merk) and the solvent system ethyl acetate: hexane (2:3). The TLC plates were visualized under UV at 260nm. Characterization of the compounds was performed through spectral analysis, including MS (Mass Spectrometry), IR, and ^{1}H-NMR. Mass spectra were recorded using a water Mass spectrometer. Infrared spectroscopy was conducted using KBr on a Simadzu IR Affinity FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker 400MHz spectrometer in DMSO solvent with TMS as an internal standard. Melting points of the synthesized compounds were determined in open glass capillary tubes and are uncorrected. The antimicrobial activities, of the synthesized compound (3a-3j), (4a-4j) have been taken by the Cup plate method, with known standard drugs utilized for comparison.

General preparation : 1-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)-phenyl)-3-(3,4-dimethoxy phenyl)prop-2-en-1-one. (3a)

To a solution containing 1-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)ethan-1-one (0.01 m) in methanol, an appropriate 3,4-dimethoxy benzaldehyde (0.01 m) was added. A catalytic amount of 40% NaOH solution was then introduced, and the resulting reaction mixture was stirred at room temperature for 24 hrs. The reaction is monitored by (TLC). After completion of the reaction, the reaction mixture was poured into crushed ice and filtered, dry it. The obtained product was crystalized in methanol, leading to the formation of the desired compounds (3a). M.P. :175ºC ; % of Yield :90%. ^{1}H NMR (400 MHz, DMSO) δ 8.37 (d, *J* = 5.7 Hz, 1H), 7.90 (d, *J* = 6.7 Hz, 1H), 7.82 (d, *J* = 7.8Hz, 1H), 7.64 (d, 1H), 7.52 (d, 1H), 7.40 (d, *J* = 7.3 Hz, 1H), 7.26 (d, 1H), 7.16 (d, *J* = 5.6 Hz, 1H), 7.08 – 6.97 (m, *J* = 8.3, 4.7 Hz, 1H), 5.32 (s, 1H), 4.92 (q, 1H), 3.84 (s, 9H), 2.23 (s, 3H). IR (cm-) 3527 (C-H Str. Aromatic), 2848 (C-H Str. Alkane), 1452-1361(C-H def. Alkane), 1581(C=C Str. Aromatic), 1107(C-H Def. Aromatic), 1649(C=O Str.), 1510(CH=CH Str. Vinyl), 1240(C-O-C Str.), 1022(C-F Str.). MS: at M/Z = 517,419,354,313,204,191,163,151,136Anal.Calcd. for $C_{27}H_{26}F_3NO_6$ C:62.66,H:5.02,O:20.88,N:2.70 Found C:62.61,H:5.00,O:20.78,N:2.66.

Similarly other (3a-3j) compounds have been synthesized.

Reaction scheme: 1



Ar = Aryl

Table : 1 Physical and analytical data of 1-(3-methoxy-4-((3-methyl-4-(2,2,2trifluoroethoxy)pyridin-2-yl) methoxy) phenyl)-3-arylprop-2-en-1-one. (3a-3j)

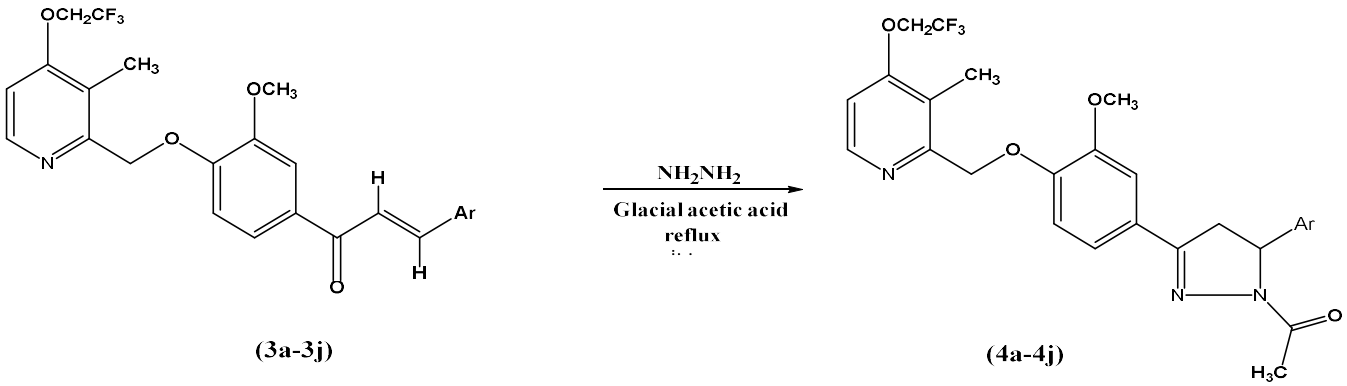
Sr. No	Ar	M.F	M. W	M.P	% Yield	% of Nitrogen	
						Calcd.	Found
3a	3,4-$(OCH_3)_2$-C_6H_3-	$C_{27}H_{26}F_3NO_6$	517	175	90	2.70	2.66
3b	4-OH,3-OCH_3-C_6H_3-	$C_{26}H_{24}F_3NO_6$	503	210	75	2.78	2.68
3c	C_6H_5-	$C_{25}H_{22}F_3NO_4$	457	155	90	3.06	3.00
3d	4-OCH_3-C_6H_4-	$C_{26}H_{24}F_3NO_5$	487	160	86	2.87	2.83
3e	4-OH-C_6H_4-	$C_{25}H_{22}F_3NO_5$	473	240	70	2.95	2.88
3f	2-NO_2-C_6H_4-	$C_{25}H_{21}F_3N_2O_6$	502	195	82	2.78	2.73
3g	3-NO_2-C_6H_4	$C_{25}H_{21}F_3N_2O_6$	502	190	78	2.78	2.75
3h	4-Cl-C_6H_4-	$C_{25}H_{21}ClF_3NO_4$	491	155	85	2.85	2.80
3i	4-Br-C_6H_4-	$C_{25}H_{21}BrF_3NO_4$	535	165	80	2.61	2.57
3j	3,4,5-$(OCH_3)_3$-C_6H_2-	$C_{28}H_{28}F_3NO_7$	547	198	64	2.55	2.53

General preparation : 3-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-5-(3,4-dimethoxy)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one: (4a)

In glacial acetic acid, a solution containing 1-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)-phenyl)-3-(3,4-dimethoxy phenyl)prop-2-en-1-one (3a) (0.01 mmol) was mixed with hydrazine hydrate (0.02 mmol). The resulting reaction mixture was refluxed at 100°C for 6 hrs. The progress of the reaction was tracked using TLC. After completion of the reaction, the reaction mixture was poured into crushed ice and filtered, dry it. The obtained product was crystalized in methanol, leading to the formation of the desired compounds (4a). M.P. : 196ºC ; % Yield : 90% ^{1}H NMR (400 MHz, DMSO) δ 8.35 (d, J = 5.6 Hz, 1H), 7.36 (s, 1H), 7.26 (d, J = 7.9 Hz, 1H), 7.20 – 7.13 (m, 2H), 6.87 (d, J = 8.3 Hz, 1H), 6.80 (s, 1H), 6.64 (d, J = 8.9 Hz, 1H), 5.47 (dd, J = 7.5 Hz, 1H), 5.22 (s, 2H), 4.92 (q, 2H), 3.80 (s, 3H), 3.72 (s, 6H), 2.30 (s, 3H), 2.21 (s, 3H), 1.25 – 1.23 (m, 2H). Anal.calcd. C:60.73, H:5.23, O:18.84, N:7.30 Found C:60.71, H:5.20, O:18.80, N:7.30. IR (cm-) 3387 (C-H Str. Aromatic), 2945 (C-H Str. Alkane), 1462-1311(C-H def. Alkane), 1516(C=C Str. Aromatic), 1294(C-H Def. Aromatic), 1649(C=O Str.), 1423(CH=CH Str. Vinyl), 1244(C-O-C Str.), 974(C-F Str.), 1006(C-N Str.), 1587(C=N Str.), 3504(N-H Str.). MS: at M/Z = 573. Anal.Calcd. for $C_{29}H_{30}F_3N_3O_6$ C:60.73,H:5.27,O:16.74,N:7.73 Found C:60.65,H:5.03,O:16.20,N:7.49.

Similarly other (4a-4j) compounds have been synthesized.

Reaction scheme : 2



(3a-3j) (4a-4j)

Ar = Aryl

Table : 2 physical and analytical data of 3-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one. (4a-4j)

Sr.No	Ar	M.F	M. W	M.P	% Yield	% of Nitrogen	
						Calcd.	Found
4a	3,4-$(OCH_3)_2$-C_6H_3-	$C_{29}H_{30}F_3N_3O_6$	573	196	90	7.33	7.30
4b	4-OH,3-OCH_3-C_6H_3-	$C_{28}H_{28}F_3N_3O_6$	559	186	77	7.51	7.49
4c	C_6H_5-	$C_{27}H_{26}F_3N_3O_4$	513	177	75	8.18	8.15
4d	4-OCH_3-C_6H_4-	$C_{28}H_{28}F_3N_3O_5$	543	185	80	7.73	7.70
4e	4-OH-C_6H_4-	$C_{27}H_{26}F_3N_3O_5$	529	250	86	7.94	7.88
4f	2-NO_2-C_6H_4-	$C_{27}H_{25}F_3N_4O_6$	558	188	72	10.03	10.02
4g	3-NO_2-C_6H_4-	$C_{27}H_{25}F_3N_4O_6$	558	190	71	10.03	10.00
4h	4-Cl-C_6H_4-	$C_{27}H_{25}ClF3N_3O_4$	547	170	84	7.68	7.60
4i	4-Br-C_6H_4-	$C_{27}H_{25}BrF_3N_3O_4$	591	195	78	7.10	7.08
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	$C_{30}H_{32}F_3N_3O_7$	603	210	66	6.96	6.92

III. RESULT AND DISCUSSION :

Antimicrobial activity :

The antibacterial and antifungal activity is done by the cup plate method through zone of inhibition in mm. The concentration of the sample & standard drug is 50 µg/ml using DMSO as solvent. The anti-bacterial activity was taken by Gram-positive bacteria Bascillis Substilis, Staphylococcus aureus & Gram-negative bacteria proteus vulgaris, Escherichia coli. The anti-fungal activity was taken by Aspergillus niger fungus. The antimicrobial activity compared with known standard drugs Streptomycin, Ampicillin, Tetracyclin and nystain. The Zone of inhibition was measured in mm. Were Zone of inhibition of compounds (3a-3j) & (4a-4j) is shown in the table No. 4 to 6.

Table : 4 Antimicrobial activity data of 1-(3-methoxy-4-((3-methyl-4-(2,2,2trifluoroethoxy)pyridin-2-yl) methoxy) phenyl)-3-arylprop-2-en-1-one. (3a-3j)

Sr. No.	Ar	Antibacterial activity, zone of inhibition in m.m.				Antifungal activity, Zone of inhibition m.m.
		Gram-positive bacteria		Gram-negative bacteria		
		B. subtilis	***S. aureus***	***E. Coli***	***P. vulgaris***	***A. niger***
3a	3,4-$(OCH_3)_2$-C_6H_3-	20	16	20	12	16
3b	4-OH,3-OCH_3-C_6H_3-	12	18	10	17	14
3c	C_6H_5-	10	20	18	15	11
3d	4-OCH_3-C_6H_4-	10	9	8	10	13
3e	4-OH-C_6H_4-	19	20	12	11	10
3f	2-NO_2-C_6H_4-	18	22	11	17	18
3g	3-NO_2-C_6H_4-	20	20	15	10	12
3h	4-Cl-C_6H_4-	19	21	8	9	19
3i	4-Br-C_6H_4-	8	20	6	14	7
3j	3,4,5-$(OCH_3)_3$-C_6H_2-	12	21	18	9	11

Table : 5 Antimicrobial activity data of 3-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one. (4a-4j)

Sr. No.	Ar	Antibacterial activity, zone of inhibition in m.m.				Antifungal activity, Zone of inhibition m.m.
		Gram-positive bacteria		Gram-negative bacteria		
		B. subtilis	*S. aureus*	*E. Coli*	*P. vulgaris*	*A. niger*
4a	3,4-$(OCH_3)_2$-C_6H_3-	24	21	22	10	9
4b	4-OH,3-OCH_3-C_6H_3-	24	10	21	11	9
4c	C_6H_5-	22	18	21	14	8
4d	4-OCH_3-C_6H_4-	24	19	22	14	9
4e	4-OH-C_6H_4-	24	20	18	13	14
4f	2-NO_2-C_6H_4-	23	19	19	14	13
4g	3-NO_2-C_6H_4-	22	20	18	11	12
4h	4-Cl-C_6H_4-	23	22	19	10	9
4i	4-Br-C_6H_4-	22	20	18	12	16
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	21	21	18	13	16

Table : 6 Compounds (3a-3j) (4a-4j) showing antibacterial & antifungal activity compared with known standard drugs:

Compound No.	Antibacterial activity, zone of inhibition in mm.				Antifungal activity, zone of inhibition in mm.
	Gram-positive bacteria		Gram-negative bacteria		
	B. subtilis	*S. aureus*	*E. Coli*	*P. vulgaris*	*A. niger*
(3a-3j)	3a,3e,3g,3h	3c,3e,3f,3g,3h, 3i,3j	3a	3b,3f,3i	3f,3h
(4a-4j)	4a,4b,4c,4d,4e,4f,4j	4a,4e, 4g,4h,4i,4j	4a,4b,4c, 4d	4c,4d,4f	4i,4j
Activity of known standard drugs:					
Drugs	*B. subtilis*	*S. aureus*	*E. Coli*	*P. vulgaris*	*A. niger*
Streptomycin	26	27	28	20	0
Ampicillin	25	26	26	19	0
Tetracycline	25	26	27	19	0
Nystatin	0	-	-	-	22

Conclusion:

We have synthesized chalcones (3a-3j) 1-(3-methoxy-4-((3-methyl-4-(2,2,2trifluoroethoxy)pyridin-2-yl) methoxy) phenyl)-3-arylprop-2-en-1-ones. The Pyrazoline derivatives (4a-4j) 3-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-ones**.** The structure confirmed by ^{1}H-NMR , IR, Mass spectra. the synthesized compounds were subjected to antibacterial & antifungal activities. Compounds **3a,3c,3e,3g,3h,3j** and **4a,4b,4c,4d,4e,4f,4g,4h,4i,4j** exhibited significant antibacterial activity against Gram-positive bacteria as compared to known standard drugs and Compounds **3a,3b,3f,3i** and **4a,4b,4c4d,4f** give remarkable activity against Gram-negative bacteria as compared to known standard drugs and Compounds **3f,3h** and **4i,4j** displayed good antifungal activity as compared to known standard drugs with the same concentration 50 µg/ml.

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REFERENCES:

1) Chithra, L., Thendral, M. T., & Shafi, S. S. (2015). Synthesis, Characterization and Antibacterial Activity of Novel Chalcone derivatives. Journal of Pharmaceutical and Biological Sciences, 3(6), 245.

2) Farooq, S., & Ngaini, Z. (2020). Synthesis, Molecular Docking and Antimicrobial Activity of α, β-Unsaturated Ketone Exchange Moiety for Chalcone and Pyrazoline Derivatives. ChemistrySelect, 5(32), 9974-9979.

3) Lodhi, G. N., Pradesh, M., & Nayak, A. (2021). Chemical and pharmacological evolution of some synthesized Chalcones and Hetrocyclic compounds,(7), 2130-2172.

4) Bhale, P. S., Dongare, S. B., & Chanshetti, U. B. (2014). Simple grinding, catalyst-free, one-pot, three-component synthesis of polysubstituted amino pyrazole. *Research Journal of Chemical Sciences*, 4(9), 16-21.

5) Lévai, A., Jekö, J., & Brahmbhatt, D. I. (2005). Synthesis of 1-substituted 5-aryl-3-(3-coumarinyl)-2-pyrazolines by the reaction of 3-aryl-1-(3-coumarinyl) propen-1-ones with hydrazines. Journal of heterocyclic chemistry, 42(6), 1231-1235.

6) Monis, r. j., chetan, s. h., & shabaraya, a. r. (2022). synthesis, characterization and biological activity studies of some substituted pyrrazolines. (11), 1316-1323

7) Asiri, A. M., & Khan, S. A. (2012). Synthesis, characterization, and in vitro antibacterial activities of macromolecules derived from bis-chalcone. Journal of Heterocyclic Chemistry, 49(6), 1434-1438.

8) Ganesh, A. (2013). Biological activities of some Pyrazoline derivatives. International Journal of Pharma and Bio Sciences, 4(2), 727-733.

9) Allison, D., Delancey, E., Ramey, H., Williams, C., Alsharif, Z. A., Al-Khattabi, H., ... & Alam, M. A. (2017). Synthesis and antimicrobial studies of novel derivatives of 4-(4-formyl-3-phenyl-1H-pyrazol-1-yl) benzoic acid as potent anti-Acinetobacter baumannii agents. Bioorganic & medicinal chemistry letters, 27(3), 387-392.

10) Chigurupati, S., Veerasamy, R., Nemala, A. R., Gowlikar, A., & Lee, K. S. (2014). Designing and in-vitro antibacterial activity of new benzotriazole compounds. World Journal of Pharmacy and Pharmaceutical Sciences, 3(3), 1645-51.

11) Bekhit, A. A., Ashour, H. M., Ghany, Y. S. A., Bekhit, A. E. D. A., & Baraka, A. (2008). Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1H-pyrazole as anti-inflammatory antimicrobial agents. European journal of medicinal chemistry, 43(3), 456-463.

12) Bhadani, V. N., Patel, P. A., Bhatt, P. V., Purohit, H. D., & Purohit, D. M. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(4), 207-216

13) El-Borai, M. A., Rizk, H. F., Abd-Aal, M. F., & El-Deeb, I. Y. (2012). Synthesis of pyrazolo [3, 4-b] pyridines under microwave irradiation in multi-component reactions and their antitumor and antimicrobial activities–Part 1. European journal of medicinal chemistry, 48, 92-96.

14) Abdellatif, K. R., Elshemy, H. A., & Azoz, A. A. (2015). 1-(4-Methane (amino) sulfonylphenyl)-3-(4-substituted-phenyl)-5-(4-trifluoromethylphenyl)-1H-2-pyrazolines/pyrazoles as potential anti-inflammatory agents. Bioorganic Chemistry, 63, 13-23.

15) Jiao, Y., Preston, S., Song, H., Jabbar, A., Liu, Y., Baell, J., ... & Gasser, R. B. (2017). Assessing the anthelmintic activity of pyrazole-5-carboxamide derivatives against Haemonchus contortus. Parasites & Vectors, 10, 1-7.

16) Chouiter, M. I., Boulebd, H., Pereira, D. M., Valentão, P., Andrade, P. B., Belfaitah, A., & Silva, A. M. (2020). New chalcone-type compounds and 2-pyrazoline derivatives: Synthesis and caspase-dependent anticancer activity. Future Medicinal Chemistry, 12(6), 493-509.

17) Ali, M. A., Yar, M. S., Siddiqui, A. A., Sriram, D., Yogeeswari, P., & De Clercq, E. (2007). Synthesis and anti-HIV activity of N'-nicotinoyl-3-(4'-hydroxy-3'-methylphenyl)-5-[substituted phenyl]-2-pyrazo-lines. Acta Pol Pharm, 64(5), 423-428.

18) Bhagwat, A. M., Bhat, A. R., Palled, M. S., Khade, A. P., & Patil, A. M. (2014). Synthesis and antihypertensive screening of novel substituted 1, 2-pyrazoline sulfonamide derivatives. Am J PharmTech Res, 4(2), 326-336.

19) Rahaman, S. A., Prasad, Y. R., Bhuvaneswari, K., & Kumar, P. (2010). Synthesis and antihistaminic activity of novel pyrazoline derivatives. Int. J. Chem. Tech. Res, 2, 16-20.

20) Umesha, K. B., Rai, K. M. L., & Nayaka, M. H. (2009). Antioxidant and Antimicrobial Activity of 5-methyl-2-(5-methyl-1, 3-diphenyl-1H-pyrazole-4-carbonyl)-2, 4-dihydro-pyrazol-3-one. International journal of biomedical science: IJBS, 5(4), 359.

21) Özdemir, Z., Kandilci, H. B., Gümüşel, B., Çalış, Ü., & Bilgin, A. A. (2007). Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. European journal of medicinal chemistry, 42(3), 373-379.

22) Amir, M., Kumar, H., & Khan, S. A. (2008). Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. Bioorganic & medicinal chemistry letters, 18(3), 918-922.

23) Barry, A. L., Hoeprich, P. D., & Saubolle, M. A. (1976). The antimicrobic susceptibility test: principles and practices.

24) Tupare, S. D. (2021). synthesis and characterization of some nitrogen containing heterocyclic derivatives via novel chalcones, (10), 1203-1213.

25) Krishnakumar, K. (2014). synthesis and characterization of some novel mannich bases of thiazolidinones derived from pyrazolines, (3), 1268-1276.

26) Hatwar, Y. A., Bhoyer, P. K., & Dixit, K. R. (2015). synthesis, characterization and biological evolution of some novel 2-pyrazoline derivatives with chalcone, (5), , 1462-1479.