ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and biological evaluation of novel series 2,4,6-trimethoxy pyrazoline.

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ABSTRACT

A novel series of compounds 2,4,6-trimethoxy pyrazoline derivatives **(4a–j)** were synthesized and characterized by ^{1}HNMR, ^{13}CNMR and mass spectroscopy. The present paper deals with newly synthesized pyrazoline compounds **(4a-j)** were screened for their antimicrobial activity against different strains of bacteria and fungi using the pour plate method. Antioxidant activity of compounds (4a-j) was carried out by DPPH and NO scavenging method. Compound **4a, 4b** and **4c** (IC_{50} = 5.2, 4 and 3.5 µg/mL) respectively had significant antibacterial profile against S. aureus (IC_{50} =0.9 µg/mL). Compound **4e** and **4h** (IC_{50} = 7.1 and 9.3 µg/mL) showed a significantly potent antifungal activity against C. albicans when compared with standard griseofulvin (IC_{50} = 6.2 µg/mL). Compound **4e** showed the better antioxidant effect i.e. (IC_{50} =5.9) in DPPH scavenging method than standard compound vitamin C (IC_{50} = 6.1 µg). Also, Compound **4i** and **4j** showed an better antioxidant effect with an IC_{50} value of 5.7 µg and 4.7g, respectively in Nitric oxide scavenging method than standard compound vitamin C (IC_{50} = 6.4 µg). Thus, the synthesized compounds **4a–j** were found to be potent antibacterial and antifungal inhibitory agents as well as an excellent antioxidant activity.

Keywords: Synthesis, Pyrazoline, 2,4,6-trimethoxy chalcone, antimicrobial and antioxidant activity.

https://doi.org/10.33887/rjpbcs/2020.11.2.4

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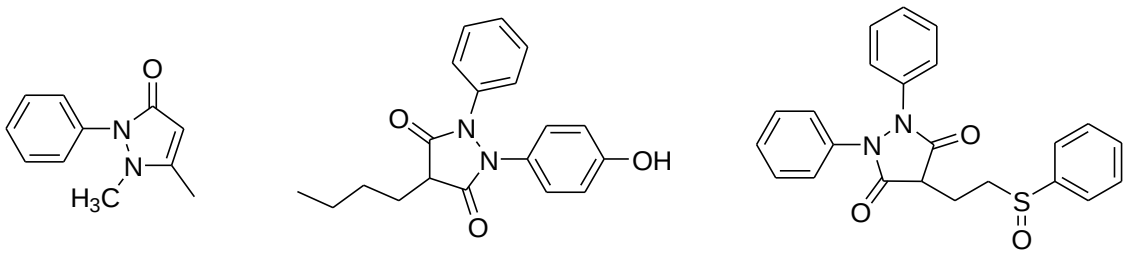
ISSN: 0975-8585

INTRODUCTION

Chalcones (1,3-diphenyl-propene-1-one) are belong to the flavonoid family [1]. Chalcones containing compounds have become of particular curiosity to chemists and researchers because of their simple synthetic procedure and wide range of different biological activities such as anti-inflammatory [2], antioxidant [3], anticancer [4] antimicrobial [5]. Chalcones containing different heterocyclic derivatives such as pyrazole, pyrazoline, pyrimidine, triazole, and Imidazole are available in the market with versatile biological activity.

Pyrazolines have occupied an important position in medicinal, pesticide and agrochemical chemistry with a wide range of biological activities such as anti-inflammatory [6], analgesic [7], antimicrobial [8], antiamoebic [9], anticancer [10] and insecticidal [11] etc. Pyrazoline motif makes up the core structure of numerous biologically active compounds, including drugs (figure1) such as Phenazone (1), Oxyphenbutazone (2) and Sulfinpyrazone (3) are some of the therapeutic drugs. Phenazone (1) is used as analgesic agent, Oxyphenbutazone (2) is used as anti-inflammatory agent and Sulfinpyrazone (3) is a used as uricosuric agent. There are different methods are available for the synthesis of pyrazoline, epoxy ketones which reacted with hydrazine and phenyl hydrazine to give pyrazolines [12], dipolar cyclo addition of nitrilimines to dimethyl fumarate and fumaro nitrile yields the corresponding pyrazolines [13] diarylidene cycloalkanones on reaction with hydrazine hydrate produce pyrazolines [14], and lastly, the most common procedure for the synthesis of 2-pyrazolines is the reaction of an aliphatic or aromatic hydrazine with α,β-unsaturated carbonyl compounds. Due to tremendous biological importance of pyrazolines moiety in the market and simple synthetic procedure, in present work; we have synthesized novel pyrazoline derivatives (4a-j) from 2,4,6-trimethoxychalcone and hydrazine hydrate having significant antibacterial, antifungal, and antioxidant activity. Also, we synthesized series of substituted pyrazoline (5a-f) from 2,4,6-trimethoxy pyrazoline and different acid or acyl chloride.



Phenazone **Oxyphenbutazone** **Sulfinpyrazone**

Figure 1 Pyrazoline containing drugs

MATERIALS AND METHODS

All of the chemicals used in this study were purchased from commercial suppliers. Melting points (m.p.) were determined in open capillary tubes and reported as the uncorrected values. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 instrument at 400 and 100MHz respectively, in $CDCl_3$ or DMSO-d_6. Chemical shifts (δ) are given in parts per million (ppm) downfield from TMS as an internal standard for ^{1H} and ^{13}C NMR. Mass spectra were recorded on Macro mass spectrometer (Waters) using electrospray ionization. The reactions were monitored by thin layer chromatography (TLC) using precoated (0.25mm) silica gel GF254 plates (E.Merck, Germany).

EXPERIMENTAL SECTION

Procedure for the synthesis of 2,4,6-trimethoxybenzaldehyde and their chalcones.

In continuation of previous research work (we mentioned process for preparation of 2,4,6-trimethoxybenzaldeyde and their chalcone (RJPBCS, 2019, 10(1), 269)). We used above charcones for further pyrazoline preparation.

ISSN: 0975-8585

General procedure for the synthesis of 2,4,6-trimethoxy pyrazoline derivatives (4).

To an ice-cold stirred solution of substituted chalcone (1.0eq) in methane (2 volume), hydrazine hydrate (5eq.) was added and stirring was continued at RT for an additional 30 mints. Then raise the reaction mixture temperature to reflux and the completion of the reaction was confirmed by TLC. Distilled off the reaction mass and cool to room temperature. Charge water and extract with dichloromethane. Dichloromethane dried over anhydrous sodium sulfate. After evaporation of the solvent under reduced pressure, a gummy mass was obtained, which solidified on treatment with petroleum ether (bp 40-60°C). Final purification of compound was done by column chromatography using silica gel (100-200 mesh), with ethyl acetate-petroleum ether (bp 40-60°C) in the ratio of 2:8 as an eluent (Scheme 1).

General procedure for the synthesis of 2,4,6-trimethoxy substituted pyrazoline derivatives (5).

To an ice-cold stirred solution of 2,4,6-trimethoxy substituted pyrazoline derivatives (1.0 eq) in dichloromethane (5vol), triethylamine (2.2 eq.) was added and stirring was continued at cold for an additional 30 mints. Chloro compound (diluted with MDC (2vol)) was added within 10-20 mints in reaction mass at 0-5 °C and stirring was continued at same temperature till the completion of the reaction was confirmed by TLC (If reaction is not completed in cold temperature then raise temperature to room temperature till the completion of the reaction was confirmed by TLC). Water charged and separated organic layer (dichloromethane). Aqueous layer further extracted with dichloromethane (2vol). The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. Final purification of the compound was done by column chromatography using silica gel (60-120 mesh), with Ethyl acetate/cyclohexane 1: 9 (Scheme 2).

4.1. Preparation of 3-(3-methoxyphenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4a)

Off-white crystalline powder yield (74.8%), m.p.: 172-173°C, $C_{19}H_{22}N_2O_4$, ^{1}H NMR(400 MHZ, $CDCl_3$) δ (ppm): 3.04 (1H, dd), 3.16 (1H, dd), 3.75 (3H, s), 3.87 (3H, s), 3.9 (6H, s), 5.14 (1H, s), 6.15 (1H, s), 6.3 (2H, d), 6.88 (1H, dd), 6.94-7.02 (2H, 7.01 m), 7.08 (1H, m).
^{13}C NMR (100 MHZ, DMSO) δ (ppm): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 162.8, 140.21, 112.35, 129.4, 115.65,128.52. MS m/z: 343.2 (M+1),

4.2. Preparation of 3-(3,4-dimethoxyphenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4b)

Off-white crystalline powder yield (70.8%), m.p.: 205-207°C, $C_{20}H_{24}N_2O_5$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.01-3.10 (2H, 3.06 (dd), 3.08 (dd)), 3.73 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 3.88 (6H, s), 5.06 (1H, dd), 6.15 (1H, s), 6.16 (2H, d), 6.64 (1H, dd), 6.72 (1H, dd), 6.96 (1H, dd). ^{13}C NMR (100 MHZ, DMSO) δ (ppm): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 162.8, 140.21, 151.23, 151.75, 109.25, 127.12, 111.12. MS m/z: 373.15 (M+1), 395.16 (M+23),

4.3. Preparation of 3-(4-methoxyphenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4c)

Off-white crystalline powder yield (68.8%), m.p.: 168-171°C, $C_{19}H_{22}N_2O_4$,^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.06 (1H, dd), 3.16 (1H, dd), 3.72 (3H, s), 3.86 (3H, s), 3.88 (6H, s), 5.14 (1H, s), 6.15 (1H, s), 6.3 (2H, d), 6.88 (1H, dd), 6.94-7.02 (2H, 7.01 m), 7.08 (1H, m). ^{13}C NMR (100 MHZ, DMSO) δ (ppm): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 162.8, 140.21, 112.35, 140.14, 115.65,130.52. MS m/z: 343.15 (M+1),

4.4. Preparation of 3-(3-nitrophenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4d)

Light yellow crystalline powder yield: 84.3%, m.p. 209-211°C, $C_{18}H_{19}N_3O_5$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.00-3.14 (1H,dd), 3.05 (1H, dd), 3.86 (3H, s), 3.87 (6H, s), 5.04 (1H), 6.15 (1H, s), 6.85 (2H, dd), 6.16 (2H, d), 6.70 (2H, dd), ^{13}C NMR ($CDCl_3$, 100 MHz): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 162.8, 140.21, 114.35, 1300.14, 115.65,160.52. MS m/z: 356.15(M-1).

4.5. Preparation of 3-(4-fluorophenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4e)

Off-wite crystalline powder yield: 78%, m.p.: 181-183°C, $C_{18}H_{19}FN_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.10-3.15 (2H, dd), 3.86 (3H, s), 3.88 (6H, s), 5.17 (1H, dd), 6.16 (1H, s), 7.07- 7.15 (4H, dd), 7.34 (2H, dd). ^{13}C NMR ($CDCl_3$, 100 MHz): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 140.21, 151.23, 153.75, 109.25, 127.12. MS m/z: 317.1 (M+1), 339.16 (M+23).

4.6. Preparation of 3-phenyl-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4f)

White crystalline powder yield: 80%, m.p.: 137-140°C, $C_{18}H_{20}N_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 2.99 (1H, dd), 3.17 (1H, dd), 3.86 (3H, s), 3.88 (6H, s), 5.17 (1H, dd), 5.85(1H, s), 6.16 (2H, d), 7.11 (2H, dd), 7.16-7.26 (3H, dd), ^{13}C NMR ($CDCl_3$, 100 MHz):): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 140.21,137, 128, 130, 140, 152.27. MS m/z: 314.14(M+2).

4.7. Preparation of 3-(4-methylphenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4g)

White crystalline powder yield: 74.8%, m.p.: 165-167°C, $C_{19}H_{22}N_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.00 (1H, dd), 3.11(1H s), 3.75 (3H, dd), 3.86 (3H, s), 3.87 (6H, s), 5.24 (1H), 6.01 (1H, s), 6.16 (2H, d), 6.90 (2H, dd), 7.07 (2H, dd). ^{13}C NMR ($CDCl_3$, 300 MHz):): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 140.21, 151.5 136, 132, 124.145.25, MS m/z: 327.1(M+1).

4.8. Preparation of 3-(4-bromophenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4h)

White crystalline powder yield: 70%, m.p.: 175-177°C, $C_{18}H_{19}BrN_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 2.95 (1H, dd), 3.17 (1H, dd), 3.86 (3H, s), 3.88 (6H, s), 5.24 (1H, dd), 5.95 (1H, s), 6.16 (2H, d), 7.35 (2H, dd), 7.48 (2H, dd).^{13}C NMR ($CDCl_3$, 100 MHz):): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 140.21, 136, 130, 131, 136.21, 158.5, 147.32. MS m/z: 391.11(M+1).

4.9. Preparation of 3-(4-chlorophenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4i)

Off-white crystalline powder yield: 80.0%, m.p.: 168-170°C, $C_{18}H_{19}ClN_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 2.95 (1H, dd), 3.17 (1H, dd), 3.86 (3H, s), 3.88 (6H, s), 5.24 (1H, dd), 5.88 (1H, s), 6.16 (2H, d), 7.35 (2H, dd), 7.48 (2H, dd). ^{13}C NMR ($CDCl_3$, 100 MHz): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 136, 132, 130, 135, 127. MS m/z: 345.21(M-1).

4.10. Preparation of 3-(2,4-dichlorophenyl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (4j)

Off-white crystalline powder yield: 80.0%, m.p.: 197-200°C, $C_{18}H_{18}Cl_2N_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.00 (1H, dd), 3.14 (1H, dd), 3.86 (3H, s), 3.87 (6H, s), 5.17 (1H, dd), 5.75 (1H, s), 6.16 (2H, d), 7.31 (1H, dd), 7.49-7.55 (2H, dd), ^{13}C NMR ($CDCl_3$, 100 MHz): 44.16, 55.85, 59.79, 131.70, 152.27, 156.28, 162.8, 130, 135, 127.165.4, 160.5 MS m/z: 379.11(M-1).

5.1 Preparation of 1-(methanesulfonyl)-5-phenyl-3-(2,4,6-trimethoxyphenyl)-4,5 dihydro-1*H*-pyrazoline (5a)

White crystalline powder yield: 80.0%, m.p.: 239-241°C, $C_{19}H_{22}N_2O_5S$, ^{1}HNMR (400 MHZ, $CDCl_3$) δ (ppm):): 2.95 (1H, dd), 3.17 (1H, dd), 3.98 (3H, s), 3.86 (3H, s), 3.88 (6H, s), 5.24 (1H, dd), 5.88 (1H, s), 6.16 (2H, d), 7.35 (2H, dd), 7.48 (2H, dd). ^{13}C NMR ($CDCl_3$, 100 MHz): 38.4, 44.16, 55.85, 59.79, 132.70, 152.27, 156.28, 169.8, 136, 132, 130, 135, 127. MS m/z: 368.31(M+1).

5.2 Preparation of 1-[5-phenyl-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (5b)

Off-white crystalline powder yield (68.8%), m.p.: 227-231°C, $C_{20}H_{22}N_2O4$,^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.01 (1H, dd), 3.17 (1H, dd), 3.94 (3H, s), 3.86 (3H, s), 3.88 (6H, s), 5.24 (1H, dd), 5.88 (1H, s), 6.16 (2H, d), 7.35 (2H, dd), 7.48 (2H, dd). ^{13}C NMR ($CDCl_3$, 100 MHz): 34.4, 44.16, 55.85, 59.79, 132.70, 152.27, 156.28, 167.8, 136, 132, 130, 135, 127. MS m/z: 455.21 (M+1), 477.13 (M+23).

ISSN: 0975-8585

5.3 Preparation of 2-chloro-1-[3-phenyl-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (5c)

Off-white crystalline powder yield (74.8%), m.p.: 255-257°C, $C_{20}H_{21}ClN_2O_4$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 2.93 (1H, dd), 3.08 (1H, dd), 3.87-3.89 (9H, 3.86 (s), 3.88 (s)), 4.22-4.23 (2H, s), 5.64 (1H, dd), 6.17 (2H, d), 7.18 (2H, ddd), 7.21 (1H, tt), 7.30 (2H, ddd).

^{13}C NMR (100 MHZ, DMSO) δ (ppm): 170.06, 158.34, 146.2, 133.28, 128.79, 121.31, 120.78, 115.01, 108, 104.75, 71.48, 59.54, 27.91 MS m/z: 399.15 (M+1).

5.4 Preparation of 1-benzyl-3-phenyl-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazoline (5d)

Crystalline powder yield (70.8%), m.p.: 200-203°C, $C_{25}H_{26}N_2O_3$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 3.01 (1H, dd), 3.08 (1H, dd), 3.88-3.89 (9H, 3.88 (s), 3.88 (s)), 5.61 (1H, dd), 6.17 (2H, d), 7.08 (2H, dtd), 7.20-7.35 (3H, 7.30 (ddd), 7.13-7.45 (4H, ddd), 7.54-7.62 (3H, 7.59 (ddd).^{13}C NMR(100 MHZ, DMSO) δ (ppm): 165.06, 158.34, 146.2, 133.28, 128.79, 121.31, 120.78, 115.01, 108, 104.75, 71.48, 59.54, 30.91 MS m/z: 403.20 (M+1).

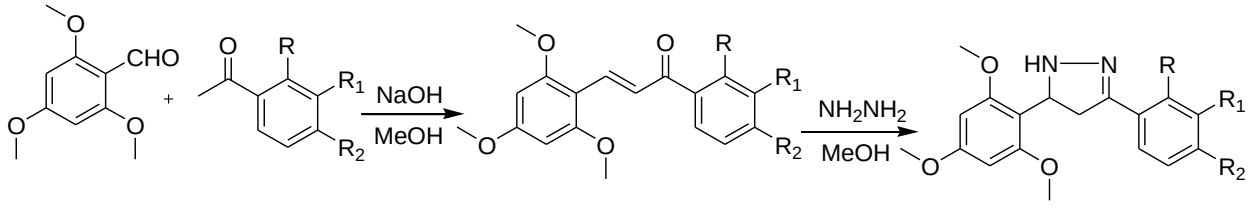
5.5 Preparation of [3-phenyl-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl (phenyl)methanone (5e)

Off-white crystalline powder yield (74.8%), m.p.: 210-213°C, $C_{25}H_{24}N_2O_4$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm): 2.85 (1H, dd), 3.02 (1H, dd), 3.86 (3H, s), 3.88 (6H, s), 4.82-4.83 (2H, 4.83 (s), 4.83 (s)), 5.31 (1H, dd), 6.15 (2H, d), 7.08-7.34 (8H, 7.29 m), 7.23 (m), 7.30 (tt), 7.21 (tt), 7.13 (dd)., ^{13}C NMR(100 MHZ, DMSO) δ (ppm): 171.06, 162.34, 146.2, 133.28, 128.79, 121.31, 120.78, 115.01, 108, 104.75, 71.48, 59.54, 31.21 MS m/z: 417.22 (M+1).

5.6 Preparation of [3-phenyl-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](4-nitrophenyl)methanone (5f)

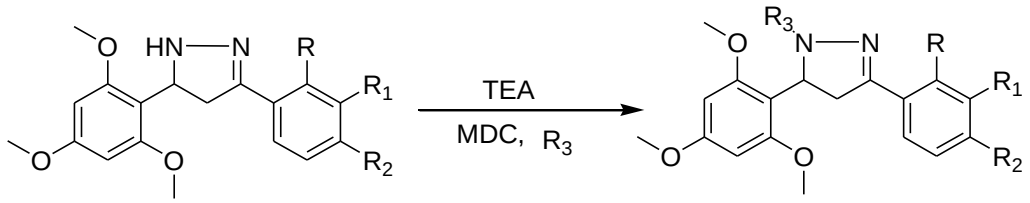
Pale yellow crystalline powder yield (74.8%), m.p.: 237-240°C, $C_{25}H_{23}N_3O_6$, ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm) 2.94 (1H, dd), 3.02 (1H, dd), 3.86 (3H, s), 3.88 (6H, s), 5.29 (1H, dd), 6.15 (2H, d), 6.70 (2H, ddd), 6.83 (2H, ddd), 7.12 (2H, dd), 7.21 (1H, tt), 7.29 (2H, dd).^{13}C NMR(100 MHZ, DMSO) δ (ppm): 167.06, 158.34, 146.2, 135.28, 128.79, 121.31, 120.78, 115.01, 108, 105.75, 71.48, 59.54, 28.91 MS m/z: 462.20 (M+1).

Reaction Scheme



1 **2** **3** **4$_{a-j}$**

Scheme I: Preparation of 2,4,6-trimethoxy pyrazoline derivatives



4 **5$_{a-f}$**

Scheme II: Preparation of substituted 2,4,6-trimethoxy pyrazoline derivatives

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A detail of substituted groups are mentioned below in table no. 1

Table 1: 2,4,6-trimethoxy pyrazoline derivatives (4a-j)

Sr. No.	Sample code	R	R_1	R_2
1	4a	H	OCH_3	H
2	4b	H	OCH_3	OCH_3
3	4c	H	H	OCH_3
4	4d	H	H	NO_2
5	4e	H	H	F
6	4f	H	H	H
7	4g	H	H	CH_3
8	4h	H	H	Br
9	4i	H	H	Cl
10	4j	Cl	H	Cl

Table 2: 2,4,6-trimethoxy Pyrazoline substituted derivatives (5a-f)

Sr. No.	Sample code	R	R_1	R_2	R_3
1	5a	H	H	H	$-SO_2CH_3$
2	5b	H	H	H	$-COCH_3$
3	5c	H	H	H	$-COCH_2Cl$
4	5d	H	H	H	$-C_6H_5$
5	5e	H	H	H	$-C_6H_5CO$
6	5f	H	H	H	$-C_6H_5NO_2$

BIOLOGICAL ACTIVITY

Antibactreial and antifungal activity evaluation

Antibacterial activity of the various synthesized compounds was evaluated by pour plate method. Briefly, the test compounds were dissolved in dimethyl sulphoxide (DMSO) to produce 1 mg/ml stock solutions. All bacterial strains were thawed, then bacteria, Staphylococcus aureus in soya broth. Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa in nutrient and Candida albicans (ATCC 1023) in potato dextrose agar medium were cultured respectively. Broth solution was prepared in bacterial cultures on media at 37±2 0C. The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish agar. To this Petri dish, 50 µL of test compounds at various concentrations (0.1 to 1000 µg/ml) were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, having a distance of 20-30 mm from another hole). The plates were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. The zone of inhibition in mm2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated. Streptomycin and gentamicin were used as standard antibacterial agents for comparison [15]

Minimum Inhibitory concentration (MIC)

The antibacterial activity of test compounds was determined by microdilution method. Test compounds were dissolved in DMSO. The 96 well plates was numbered as per the test compounds and the microorganism used, to which 50µl of various concentrations of test compounds were added to this, 50µl of test organism suspension was added. To this inoculated broth was added. The plate was sealed and incubated for 24 hr at room temperature.

ISSN: 0975-8585

RESULTS

IC_{50} values for antibacterial activity were determined using standard agar method using Streptomycin as standard for gram positive bacteria and Gentamycin as standard for gram negative bacteria. From activity data (Table 3), a comparison of antibacterial activity of compounds with that standard drug Streptomycin (IC_{50} = 0.9 µg/mL) showed that –OCH_3 at the *meta, para and meta & para* position in compound **4a, 4b and 4c** (IC_{50} = 5.2, 4 and 3.5 µg/mL) respectively had significant antibacterial profile against *S. aureus*. Also, compound **4a, 4b and 4c** (IC_{50} = 2.5, 1.7 and 3.2 µg/mL) respectively had moderate antibacterial profile against *B. substalis* (IC_{50} = 0.85 µg/mL). As observed from activity data (Table 3), compounds **4a, 4b and 4c** with electron donating group like –OCH_3 on the aromatic group were more active than electron withdrawing groups like –CH_3, -X (Halide =F, Br, Cl) on the aromatic group.

Table 3: Antimicrobial activity of the compounds (4a-j)

Test compounds	S. aureus		B. substalis		P. auroginosa		E. Coli		Candida albicans	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
4a	5.2		2.5	10	23.2	10	8.7	1	85.2	10
4b	3.5	10	1.7	1	11.5	10	5.1	1	51.7	10
4c	4	10	3.2	1	17.7	10	6.6	1	48.2	10
4d									13.5	10
4e	5.2	10	7.3	10	34.7	10			7.1	10
4f	9.8	10	13.2	1	50.1	10	42.3	10	16.5	10
4g	15	1	17.2	1	54.2	10	41.5	10	25.5	10
4h	21	1	23.7	1	74.3	1	21.7	10	9.3	10
4i	32.3	1	16.5	1	85.9	1	27.5	10	11.1	10
4j	45.2	1	32.2	1	88.5	1	31.2	10	15.1	10
Stretomycin	0.9	0.1	0.85	0.1	--	-	-		-	-
Gentamycin	-	-	-	-	6.1	0.1	4.95	0.1	-	-
Griseofulvin	-	-	-	-	-	-	-	-	6.5	0.1

Antifungal activity

Inhibitory concentration (IC_{50}) values for antifungal activity against *C. albicans* were determined using standard agar method using Griseofulvin as standard. Dimethyl sulfoxide was used as solvent control. The results of *in vitro* antifungal activity (Table 3) showed that synthesized compounds **4a–j** have moderate to good activity. Also, our results demonstrated that most potent **4e and 4h** (IC_{50} = 7.1 and 9.3 µg/mL) showed a significantly potent antifungal activity against *C. albicans* when compared with standard griseofulvin (IC_{50} = 6.2 µg/mL). Comparison of antifungal activity of compounds with that standard drug griseofulvin (IC_{50} = 6.2 µg/mL) showed that -F and -Br at the *meta* position at compound **4e and 4h** had more antifungal activity then the compound substituted with –OCH_3 at meta and para position. Structure–activity relationship (SAR) revealed that compounds **4e** and **4h** with electron withdrawing groups were more active than compounds **4a, 4b** and **4c** with electron donating groups.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity

The free radical scavenging activity of test compounds was determined by DPPH scavenging method as per the procedure described earlier by Shen [16]. In this method, 0.1mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution, 1.5 mL of test compounds of the dissolved in DMSO were added at various concentrations of all (1, 10, 100, 500 & 1000 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Shimadzu, spectrophotometer). Vitamin C was used as a standard compound. Reduction in absorbance by test compounds and indicates radical scavenging activity. The scavenging activity by the DPPH radical was determined by

DPPH scavenging effect (% inhibition) = {(A0 -A1)/A0) ×100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance test compound and vitamin C.

Compound **4e** and **4h,** showed the excellent antioxidant effect with an IC_{50} value of, 5.9 µg, and 6.5 µg respectively in DPPH scavenging method (Table 4), that is better than and equivalent to standard compound vitamin C (IC_{50} = 6.1 µg) respectively. Compound **4d, 4f** and **4i** showed the antioxidant effect with an IC_{50} value of 7.1 µg, 7.8µg and 6.8µg, respectively (Table 4), which is promising activity with a comparison with standard compound vitamin **C** (IC_{50} = 6.1 µg).

Nitric oxide (NO) radical scavenging Activity

Nitric oxide (NO) radical scavenging activity of various test compounds was determined as per the procedure described by Balakrishnan [17]. Briefly, various concentrations of test compounds (as 1, 10, 100, 500, and 1000 µg/ml) were prepared in ethanol. To 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline, to this, 1 ml of various concentrations of test compounds were mixed, and to this equal volume of freshly prepared Griess reagent was added, solution was then incubated at 25°C for 3 hours. Form this, 100 µl of the reaction mixture was transferred to a 96-well plate, and the absorbance was read at 546 nm using a microplate reader (Biotek, Italy). Ascorbic acid was used as a standard control.

The percentage of nitrite radical scavenging activity of test compounds was calculated by

$$\text{Nitric oxide scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test compounds}}{\text{Absorbance of control}} \times 100$$

Compound **4i** and **4j** showed the excellent antioxidant effect with an IC_{50} value of 5.7 µg and 4.7g, respectively in Nitric oxide scavenging method (Table 4), that is better than standard compound vitamin C (IC_{50} = 6.4 µg). Compound **4h** and **4f** showed the promising antioxidant effect with an IC_{50} value of 8.1 µg and 10 µg, respectively (Table 4), which is promising activity with a comparison with standard compound vitamin **C** (IC_{50} = 6.4µg).

Table 4: Antioxidant activity of the compounds (4a-j)

Test compounds	Concentration (µg/mL)	IC_{50} (µg/mL)	
		DPPH	NO
4a	1-1000	28.6	38
4b	1-1000	21.4	31
4c	1-1000	12.3	41.3
4d	1-1000	7.1	21.2
4e	1-1000	5.9	36.1
4f	1-1000	7.8	10
4g	1-1000	11.2	
4h	1-1000	6.5	8.1
4i	1-1000	6.8	5.7
4j	1-1000	326	4.7
Vitamin C	1-1000	**6.1**	**6.4**

CONCLUSION

In summary, we have synthesized novel 2,4,6-trimethoxy pyrazoline derivatives **(4a-j)** and antibacterial, antifungal and antioxidant activities of all the newly synthesized compounds were studied. Also we further prepared substituted pyrazoline derivative. The antibacterial activity for compound **4a, 4b** and **4c** (IC_{50} = 5.2, 4 and 3.5 µg/mL) respectively had significant antibacterial profile against *S. aureus*. Also, compound **4a, 4b** and **4c** (IC_{50} = 2.5, 1.7 and 3.2 µg/mL) respectively had moderate antibacterial profile against *B. substalis* (IC_{50} = 0.85 µg/mL). For antifungal activity, compound **4e** and **4h** (IC_{50} = 7.1 and 9.3 µg/mL) showed a significantly potent antifungal activity against *C. albicans* when compared with standard griseofulvin (IC_{50} = 6.2

µg/mL). Compound **4e** and **4h,** showed the excellent antioxidant effect with an IC_{50} value of, 5.9 µg, and 6.5 µg respectively in DPPH scavenging method, that is better than and equivalent to standard compound vitamin C (IC_{50} = 6.1 µg) respectively. Other compound **4d, 4f** and **4i** showed the promising antioxidant effect with an IC_{50} value of 7.1 µg, 7.8µg and 6.8µg, respectively. Also, Compound **4i** and **4j** showed the excellent antioxidant effect with an IC_{50} value of 5.7 µg and 4.7g, respectively in Nitric oxide scavenging method, that is better than standard compound vitamin C (IC_{50} = 6.4 µg). Other compound **4h** and **4f** showed the promising antioxidant effect with an IC_{50} value of 8.1µg and 10µg, respectively (Table 4), Thus, the synthesized compounds **4a–j** were found to be potent antibacterial and antifungal inhibitory agents as well as an excellent antioxidant activity. Hence, the newly synthesized compound can serve as an important gateway for the design and development of new good oral drug-like antimicrobial and antioxidant agents. Lastly, there are new opportunities for the possible modification as per the pharmaceutical requirement in the future. The present investigation has provides impetus for development of more potent 2,4,6-trimethoxy chalcone substituted different heterocyclic derivatives with different biological activity.

ACKNOWLEDGEMENTS

The authors are gratefully acknowledging the support provided by Nirma University, Ahmedabad.

REFERENCES

[1] Yau-Hung C, Wei-Hua W, Yun-Hsin W, Zi-Yu L, Chi-Chung W, Ching-Yuh C. Evaluation of the Anti-Inflammatory Effect of Chalcone and Chalcone Analogues in a Zebrafish Model Molecules 2013; 18: 2052-2060.
[2] Go ML, Wu X, Liu X. Curr Med Chem 2005; 12: 483–499.
[3] Liu, X-F, Zheng, C.-J, Sun, L.-P, Liu, X-K,Piao, H-R. Eur J Med Chem 2011; 46: 3469.
[4] Dimmock J, Elias D, Beazely M, Kandepu N. Curr Med Chem 1999; 6: 1125–1149.
[5] Zoldakova M, Kornyei Z, Brown A, Biersack B,Madarász E,Schobert R. Biochem Pharmacol 2010; 80: 1487.
[6] Joshi, R. S.; Mandhane, P. G.; Diwakar, S. D.; Dabhade, S. K.; Gill, C. H. Bioorg. Med. Chem. Lett. 2010, 20, 3721-3725.
[7] Khode, S.; Maddi, V.; Aragade, P.; Palkar, M.; Ronad, P. K.; Mamledesai, S.; Thippeswamy, A. H.; Satyanarayana, D. Eur. J. Med. Chem. 2009, 44, 1682-1688.
[8] Sivakumar, P. M.; Ganesan, S.; Veluchamy, P.; Doble, M. Chem. Biol. Drug Des. 2010, 76, 407- 411.
[9] Adhikari, N.; Maiti, M. K.; Jha, T. Bioorg. Med. Chem. Lett. 2010, 20, 4021-4026.
[10] Havrylyuk, D.; Kovach, N.; Zimenkovsky, B.; Vasylenko, O.; Lesyk, R. Arch. Pharm. Chem. life Sci. 2011, 344, 514-522.
[11] Zhao, P. L.; Wang, F.; Zhang, M. Z.; Liu, Z. M.; Huang, W.; Yang, G. F. J. AgriFood Chem. 2008, 56, 10767-10773.
[12] M. A. El. Hashah, M. El-Kady, M. A. Saiyed, A. A. Elsawy; Egypt. J. Chem., 27(6), 715-21 (1985) (Eng.); Chem. Abstr., 105, 20868u (1986).
[13] M. Hassaneen Hamdi, A. Ead Hamad, A. H. Mousa Hiyam; Sulfur Lett. 8(5), 27582 (1989); Chem. Abstr., 111, 5761l (1989).
[14] R. A. Kabli, A. M. Kaddah, A. M. Khalil, A. A. Khalaf; Indian J. Chem., 25(B), (2), 152-6 (1986); Chem. Abstr. 106, 11975b (1987).
[15] Vincent J, Vincent H. Proc. Soc. Exp. Biol. Med. 1944; 55: 162–164.
[16] Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity in vitro of selenium-contained protein from the se-enriched. Bifodobacterium animalis 01.Anaerobe, 2010; 16: 380-386.
[17] Balakrishnan N, Panda AB, Raj NR, Shrivastava A., Prathani R. The evaluation of nitric oxide scavenging activity of Acalypha indica Linn root. Asian Journal of Research in Chemistry 2009; 2: 148-150.