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Synthesis and anti-bacterial, anti-fungal activity of novel pyrazoline derivatives containing phenothiazine ring

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Abstract
A Novel Series of Compounds were synthesised by Condensation reaction of phenothiazine aldehyde with Substituted Acetophenone derivatives 4 (a-d) in presence of alcoholic Sodium hydroxide Solution to get Chalcone Intermediates 5 (a-d), which were further treated Hydrazine hydrate in Ethanol to get Novel Pyrazoline Derivatives 6 (a-g). The Structures of new compounds were confirmed by IR and ^{1}H NMR and ^{13}C NMR Spectral Data. When these compounds were evaluated for Anti-bacterial and Anti-Fungal Activity, Some of them were found to possess Significant Activity, When Compared to Standard Drugs.

Keywords: Chalcone, Pyrazolines, Antibacterial, Antifungal Activity

Introduction
Heterocyclic compounds have so far been synthesized mainly due to the wide range of biological activities. Much attention has paid to the synthesis of Heterocyclic compounds bearing nitrogen atoms containing ring system, like Pyrazoline mainly due to their higher pharmacological activity. Heterocyclic Compounds have so far been Synthesized Mainly due to the wide range of Biological Activities [1]. The review of the literature shows that the pyrazoline nucleus is quite stable and has inspired chemists to utilize these stable fragments in bioactive moieties to synthesize new compounds possessing biological activities [2-4]. The past studies of substituted pyrazolines revealed that they exhibit antibacterial [5], analgesic [6], anti-inflammatory [7], anti-viral [8], anti-fungal [9], anti-arthritic [10], cerebroprotective effect [11], and anti-depressant [12] properties. There are several substituted pyrazolines having bleaching property or act as luminescent and fluorescent agents [13]. They are also useful as biodegradable agrochemicals [14].
A variety of substituted pyrazolines [15] and their derivatives are vital biological agents and significant amounts of research action have been directed in the direction of this class of compounds. Nitrogen containing five membered heterocyclic compounds, natural in addition to synthetic, have been received significant attention due to the broad range of pharmacological activities. Pyrazoline shows one of the energetic classes of compounds associating a broad spectrum of biological activities. Pyrazoline has been reported to acquire anti-diabetic [16], anti-diuretic [17], anti-analgesic [18], anti-helimentic [19], anti-hypolipaemic [20], anti-malarial [21], and anti-depressant [22] activities.
Encouraged by the diverse biological activities of pyrazoline compounds, it was decided to prepare a new series of Pyrazoline derivatives. These derivatives contains phenothiazine nucleus. Literature survey revealed that incorporation of phenothiazine ring in Pyrazoline Heterocyclic ring enhanced antibacterial and antifungal activity. In the present communication, chalcones (5a-g) were prepared by the action of substituted acetophenone derivatives (4 a-g) with phenothiazine aldehyde in the presence of aqueous solution of Sodium hydroxide and Ethanol at room temperature by Claisen-Schmidt condensation method. The synthesized chalcones further condensed with Hydrazine hydrate in presence of pyridine to obtained Pyrazoline derivatives (6a-g). The synthesis of the compounds as per the following Scheme I given below.
The synthetic route was depicted in scheme I
The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H NMR spectral data and Elemental analysis. Further these compounds were subjected for antifungal and antibacterial activity.

Materials and Methods:

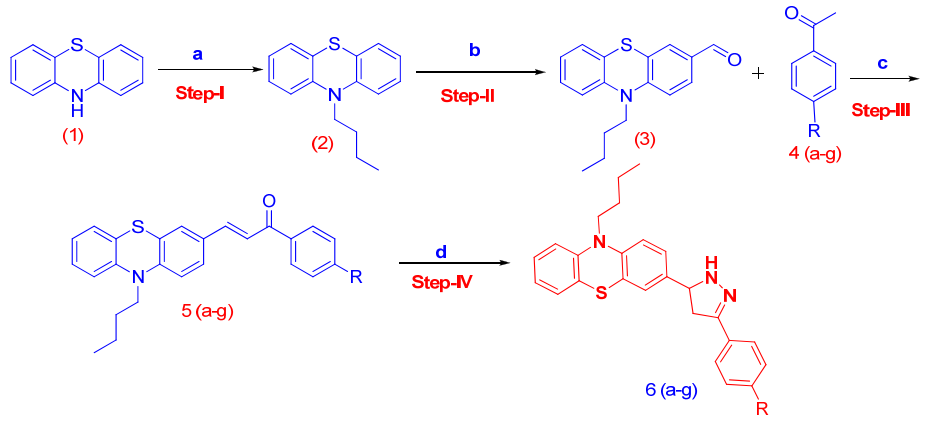
Laboratory chemicals were provided by Rankem India Ltd. and Ficher Scientific Ltd. Melting points were determined by the open tube capillary method and are not correct. The purity of the compounds was determined by thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene: ethyl acetate (8:2). The spots were observed by exposure to iodine Vapours or by UV light or P-Anisaldehyde Stain Solution. The IR spectra were received by PerkinElmer 1720 FT-IR spectrometer (KBr pellets). The ^{1}H NMR &^{13} C NMR spectra were obtained by Bruker Advance II 400 spectrometer using TMS because the internal standard in $CDCl_3$. Elemental analysis of the new synthesized compounds were obtained by Carlo Erba 1108 analyzer. General Information. Commercial chemicals were treated as follows: DMF, distilled from CaH_2 and degassed (freeze and thaw) three times prior to use; THF, ether, hexanes distilled from Na/benzophenone.

The synthesis of the compounds as per the following Scheme I given below.

The synthetic route was depicted in scheme I

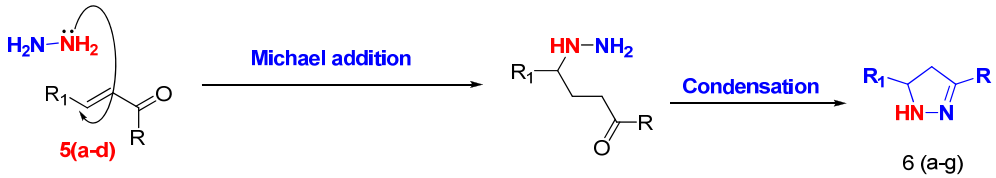
The title compounds 6(a-g) were synthesised in Four sequential steps using different reagents and reaction conditions, the 6(a-g) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data.

SYNTHETIC SCHEME : I



Reagents and Reaction conditions: (a) Butyl bromide, DMSO, NaOH,100^{0}C **(b)** DMF,$POCl_3$,1,2 DCE,Reflux **(c)**KOH,Ethanol, RT **(d)** Ethanol, Hydrazine hydrate, Reflux

Possible Mechanism For Formation of Pyrazoline Heterocyclic ring 6(a-g) Formation :



R_1 = phenothiazine A Ring
R= Aromatic Ketones

Experimental Section

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. THF was distilled from sodium benzophenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20–30 Torr). Flash chromatography was performed with silica gel (200–300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for ^{1}H for ^{13}C, respectively, in $CDCl_3$ solution with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded using tetramethylsilane (TMS) in the solvent of $CDCl_3$-d or DMSO-d_6 as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for the preparation of 10-butyl-10H-phenothiazine Compound (2) [23]

To a solution of 1 (0.1 m.mol) in dry DMSO (5 ml) was added 1-bromobutane (0.15 m.mol) and NaOH (1 m.mol). The solution was refluxed under stirring during 8 h, neutralised with HCl (2N, 7 ml) and methylene chloride (20 ml) was

added. The organic phase was separated, washed twice with water (40 ml) and evaporated. to give a yellow oil (3)
Yield :58%.
^{1}H-NMR (400 M.HZ, DMSO-d_6): δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.3(8H,m,Ar-H)
IR(KBr,cm-^{1}): 3110 cm^{-1} (Ar C-H stret), 1550 cm^{-1} (C=C Stret), 2900 (SP^3 C-H Stretch) Wave numbers respectively.

General procedure for the preparation of 10-butyl-10H-phenothiazine-3-carbaldehyde (Compound 3) [24]

Introduce 8.5 ml (110 m. mol) of dry Dimethyl formamide, Cool the dimethylformamide (DMF) and add over 30 minutes 2.61 ml (28 m, mol) of phosphoryl chloride($POCl_3$),Then add, over 40 minutes, the solution of 3 g (25.5 m.mol) of compound(2) in 5 ml of anhydrous 1,2 Di chloro Ethane, making sure that the temperature does not rise above 10 ^{0}C. Stir the` mixture for 45 minutes at 10 ^{0}C. then for 40 minutes at 85 ^{0}C. Add 10 g of crushed ice, stir the compact mixture vigorously and add a further 10 g of crushed ice. Continue the stirring and add progressively, by a dropping funnel, a solution of 11.3 g (282 m.mol) of sodium hydroxide in 30 ml of water, slowly at first, then more rapidly, maintaining a good level of stirring. Then bring the solution to the boil for 15 minutes, recover by filtration and wash the isolated semi solid several times with water.
Yield: 70%
^{1}H-NMR (400 M.HZ, DMSO-d_6): δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.3(7H,m,Ar-H), 9.2 (1H,S, H-C=O)
IR(KBr,cm-^{1}): 3110 cm^{-1} (Ar C-H stret), 1550 cm^{-1} (C=C Stret), 2900 (SP^3 C-H Stretch), 1725 cm^{-1} (C=O Stretch) Wave numbers respectively.

General procedure for the preparation of (E)-3-(10-butyl-10H-phenothiazin-3-yl)-1-(4-nitro/Methoxy/Methyl/Fluoro/Chloro/Bromo phenyl)prop-2-en-1-one (5 a-f), (E)-3-(10-butyl-10H-phenothiazin-3-yl)-1-phenylprop-2-en-1-one (5g): [25]

10-butyl-10H-phenothiazine-3-carbaldehyde (Compound 3) (5 m.mol) and Acetophenone derivatives 4(a-g) (5 m.mol) were dissolved in Ethanol (10 ml) with stirring. Potassium hydroxide (70%) (15 m.mol) was added in portions to give a blood-red solution. Resulting solution was stirred for 8–28h, during which Corres [onding chalcone precipitated as the potassium salt. The solution/suspension was poured into cold 2 N HCl (10 ml), and further concentrated HCl was added until the solution was acidic. The resulting yellow solid was filtered, washed with water (20 ml), and recrystallized from corresponding solvent (MeOH or MeOH/CH_2Cl_2) to give the products 5(a-g).

Table 1: Yields & Melting Points of Corresponding Compounds (5 a-g)

S. No	Yield (%)	Melting Point (^{0}C)
5a	75	111-112
5b	72	183-184
5c	70	126-127
5d	76	143-144
5e	73	115-116
5f	71	124-126
5g	78	90-91

Table 2: IR(KBr,cm-^{1}) data of Compounds 5 (a-g)

Compound	ν_{max}, cm^{-1}
5a	3110 cm^{-1} (Ar C-H stret), 1610 cm^{-1} (C=C Stret), 2900 (SP^3 C-H Stretch), 1525 & 1350 cm^{-1} (two bands,N-O Stretch in –NO_2 Group) Wave numbers respectively.
5b	3100 cm^{-1} (Ar C-H stret), 2910 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1150 (C-O-C Stretch), Wave numbers respectively.
5c	3110 cm^{-1} (Ar C-H stret), 290 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), Wave numbers respectively.
5d	3110 cm^{-1} (Ar C-H stret), 2900 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1368 cm^{-1} (C-F Stretch) Wave numbers respectively.
5e	3110 cm^{-1} (Ar C-H stret), 2940 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 768 cm^{-1} (C-Cl Stretch), 770 cm^{-1} (C-Cl Stretch) Wave numbers respectively.
5f	3110 cm^{-1} (Ar C-H stret), 2920 cm^{-1} (SP^3 C-H Stretching), 1580 cm^{-1} (C=C Stret), 568 cm^{-1} (C-Br Stretch) Wave numbers respectively.
5g	3120 cm^{-1} (Ar C-H stret), 2940 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), Wave numbers respectively.

Table 3: ^{1}H –NMR data of Synthesised compounds 5(a-g)

Compound	^{1}H-NMR ($CDCl_3$-d_1) (δ ppm)
5a	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.3(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 8.1(2H,d,J=8HZ, meta to nitro group), 8.5(2H,d,J=8HZ, ortho to nitro group).
5b	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 8.2(2H,d,J=8HZ, meta to Methoxy group), 7.2(2H,d,J=8HZ,ortho to Methoxy group), 3.9(3H,S, -OCH_3)
5c	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 8(2H,d,J=8HZ, meta to Methyl group), 7.5(2H,d,J=8HZ,ortho to Methyl group), 2.3(3H, S, -CH_3)
5d	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 7.9(2H,d,J=8HZ, meta to Fluoro group), 7.5(2H,d,J=8HZ, ortho to Fluoro group)
5e	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 7.9(2H,d,J=8HZ, meta to chloro group), 7.7(2H,d,J=8HZ,ortho to Chloro group)
5f	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 8(1H,d,J=14HZ,Beta alkene Proton from carbonyl group), 7.5(1H, d, J=14Hz,alpha alkene proton from carbonyl group), 7.9(2H,d,J=8HZ, meta to Bromo group), 7.8(2H,d,J=8HZ,ortho to Bromo group)
5g	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.7(12H,m,Ar-H)

General procedure for the preparation of 10-butyl-3-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6a), 10-butyl-3-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6b), 10-butyl-3-(3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6c), 10-butyl-3-(3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6d), 10-butyl-3-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6e), 3-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10-butyl-10H-phenothiazine (6f), 10-butyl-3-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6g) : [26]

To a Solution of Compound (5a-5g) (0.01 m.mol) and 98% hydrazine hydrate (0.02 m.mol) in absolute Ethanol and add few drops of hydro chloric acid. The reaction mixtures were refluxed for 8-10 hrs, distilled in vacuum and cooled. The Seperated Solids Were filtered and washed with ether and recrystallised from Ethanol solvent.Physical, analytical and spectroscopic data of compounds (6a-6g) as follows, respectively.

10-butyl-3-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6a)

Yellowish crystals, Yield 60%, m.p. 168 ^{o}C; TLC (Acetone: Pet.ether, (2:8).

10-butyl-3-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6b)

Yellowish crystals, Yield 50%, m.p. 154^{o}C; TLC (Acetone: Pet.ether, (2:8).

10-butyl-3-(3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6c)

Yellowish crystals, Yield 55%, m.p. >300^{o}C; TLC (Acetone: Pet.ether, (2:8).

10-butyl-3-(3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6d)

Greenish crystals, Yield 58%, m.p. 162 ^{o}C; TLC (Acetone: Pet.ether, (2:8).

10-butyl-3-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6e)

Shiny greenish crystals, Yield 54%, m.p. 214 ^{o}C; TLC (Acetone: Pet.ether, (2:8).

3-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-10-butyl-10H-phenothiazine (6f)

greenish crystals, Yield 55%, m.p. 114 ^{o}C; TLC (Acetone : Pet.ether, (2:8).

10-butyl-3-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-10H-phenothiazine (6g)

Slight Yellowish crystals, Yield 50%, m.p. 184 ^{o}C; TLC (Acetone: Pet.ether, (2:8).

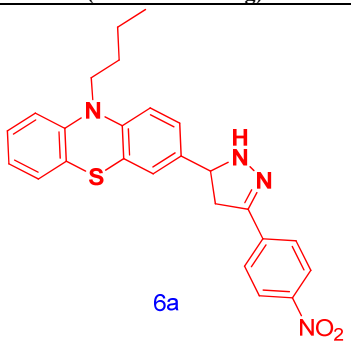
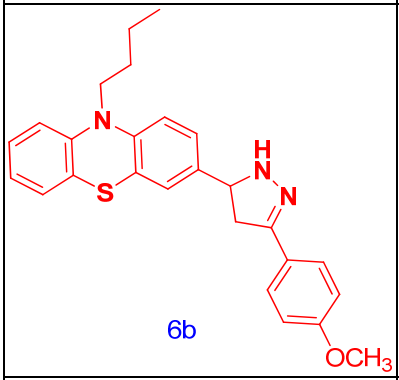
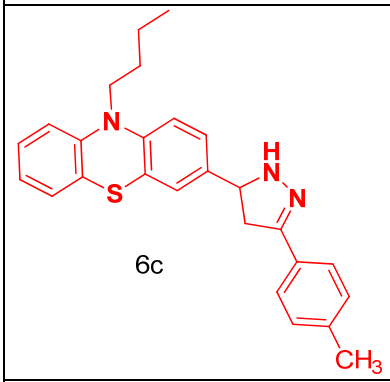
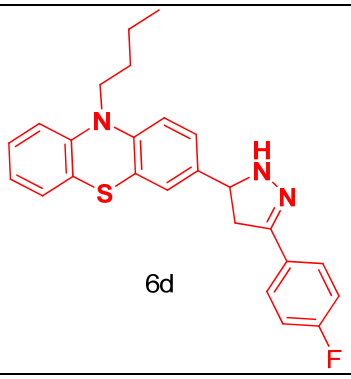
Table 4: IR(KBr,cm-^{1}) data of Compounds 6 (a-g)

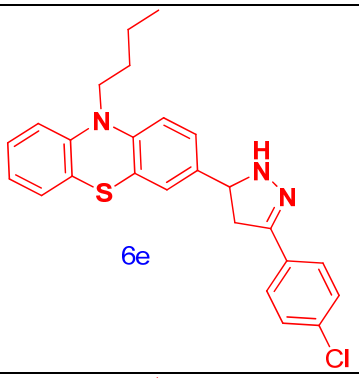
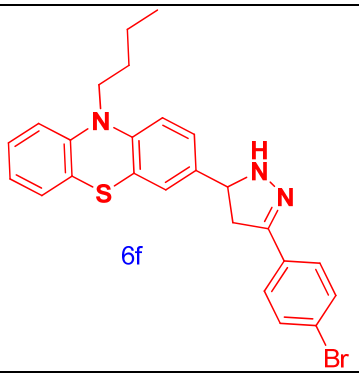
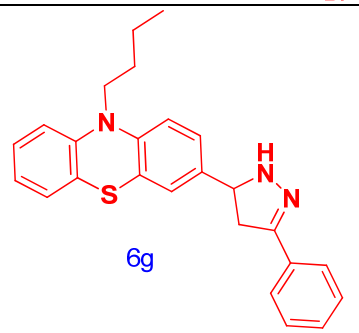
Compound	ν_{max}, cm^{-1}
6a	3410 (N–H), 3080 cm^{-1} (Ar C-H stret), 1610 cm^{-1} (C=C Stret), 2900 (SP^3 C-H Stretch), 1525 & 1350 cm^{-1} (two bands,N-O Stretch in –NO_2 Group), 1630 (C=N) in pyrazoline ring, 1580 (N-N) in pyrazoline ring, 1380 (C–N).Wave numbers respectively.
6b	3420 (N–H), 3070 cm^{-1} (Ar C-H stret), 2910 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1150 (C-O-C Stretch), 1610 (C=N) in pyrazoline ring, 1520 (N-N) in pyrazoline ring, 1360 (C–N).Wave numbers respectively.
6c	3430 (N–H), 3020 cm^{-1} (Ar C-H stret), 290 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1580 (C=N) in pyrazoline ring, 1510 (N-N) in pyrazoline ring, 1330 (C–N).Wave numbers respectively.
6d	3415(N–H), 3080 cm^{-1} (Ar C-H stret), 2900 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1368 cm^{-1} (C-F Stretch), 1600 (C=N) in pyrazoline ring, 1520 (N-N) in pyrazoline ring, 1300 (C–N). Wave numbers respectively.
6e	3420 (N–H), 3070 cm^{-1} (Ar C-H stret), 2940 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 768 cm^{-1} (C-Cl Stretch), 1630 (C=N) in pyrazoline ring, 1580 (N-N) in pyrazoline ring, 1380 (C–N). Wave numbers respectively.
6f	3415 (N–H), 3060 cm^{-1} (Ar C-H stret), 2920 cm^{-1} (SP^3 C-H Stretching), 1580 cm^{-1} (C=C Stret), 568 cm^{-1} (C-Br Stretch), 1640 (C=N) in pyrazoline ring, 1570 (N-N) in pyrazoline ring, 1390 (C–N). Wave numbers respectively.
6g	3420 (N–H), 3120 cm^{-1} (Ar C-H stret), 2940 cm^{-1} (SP^3 C-H Stretching), 1550 cm^{-1} (C=C Stret), 1630 (C=N) in pyrazoline ring, 1580 (N-N) in pyrazoline ring, 1380 (C–N). Wave numbers respectively.

Table 5: ^{1}H –NMR data of Synthesised compounds 6(a-g)

Compound	^{1}H-NMR ($CDCl_3$-d_1) (δ ppm)
6a	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.3(7H,m,Ar-H), 7.25 (s, 1H, NH in pyrazoline ring), 3.7-3.94 (dd, 2H, CH_2 in pyrazoline ring), 3.9(1H,dd, 1H in Pyrazoline ring), 8.1(2H,d,J=8HZ, meta to nitro group), 8.5(2H,d,J=8HZ, ortho to nitro group).
6b	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 7.15 (s, 1H, NH in pyrazoline ring), 3.7-3.96 (dd, 2H, CH_2 in pyrazoline ring), 3.9(1H,dd, 1H in Pyrazoline ring), 8.2(2H,d,J=8HZ, meta to Methoxy group), 7.2(2H,d,J=8HZ,ortho to Methoxy group), 3.9(3H,S, -OCH_3)
6c	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 7.10 (s, 1H, NH in pyrazoline ring), 3.7-3.94, (dd, 2H, CH_2 in pyrazoline ring), 3.8(1H,dd, 1H in Pyrazoline ring), 8(2H,d,J=8HZ, meta to Methyl group), 7.5(2H,d,J=8HZ,ortho to Methyl group), 2.3(3H, S, -CH_3)
6d	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 7.10 (s, 1H, NH in pyrazoline ring), 3.7-3.94, (dd, 2H, CH_2 in pyrazoline ring), 3.8(1H,dd, 1H in Pyrazoline ring), 7.9(2H,d,J=8HZ, meta to Fluoro group), 7.5(2H,d,J=8HZ,ortho to Fluoro group)
6e	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 7.10 (s, 1H, NH in pyrazoline ring), 3.7-3.94, (dd, 2H, CH_2 in pyrazoline ring), 3.8(1H,dd, 1H in Pyrazoline ring), 7.9(2H,d,J=8HZ, meta to chloro group), 7.7(2H,d,J=8HZ,ortho to Chloro group)
6f	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7-7.2(7H,m,Ar-H), 7.10 (s, 1H, NH in pyrazoline ring), 3.7-3.94, (dd, 2H, CH_2 in pyrazoline ring), 3.8(1H,dd, 1H in Pyrazoline ring), 7.9(2H,d,J=8HZ, meta to Bromo group), 7.8(2H,d,J=8HZ,ortho to Bromo group)
6g	δ 0.90 (t, J = 7.3 Hz, 3H), 1.40 (m, 2H), 1.49 (m, 2H), 3.90 (t, J = 7.0 Hz, 2H), 7.10 (s, 1H, NH in pyrazoline ring), 3.7-3.94, (dd, 2H, CH_2 in pyrazoline ring), 3.8(1H,dd, 1H in Pyrazoline ring), 7-7.7(12H,m,Ar-H).

Table 6: ^{13}C –NMR data of of Novel Synthesised compounds 6(a-g):

Structure of the compound (With numbering)	^{13}CNMR (100 M.HZ, DMSO-d_6, δ ppm)
 6a	150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons) respectively.
 6b	115-150 (19 Aromatic carbons), 50 & 44 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons), 55 (-O-**C**H_3) respectively.
 6c	115-150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons), 21 (Aromatic Methyl Carbon) respectively.
 6d	115-150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons) respectively.

 6e	115-150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons) respectively.
 6f	115-150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons) respectively.
 6g	115-150 (19 Aromatic carbons), 50 & 43 (Pyrazoline ring Carbons) 48(N-**C**H_2), 13-25(3 aliphatic carbons) respectively.

Pharmacological results

Anti-bacterial activity

All the synthesized compounds were screened for their in vitro antibacterial activity. Bacillus megaterium, Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Eschericha coli, Enterobacter, Proteus vulgaris and Pseudomonas aeruginosa strains were used to determine antibacterial activity in which first four are gram positive bacteria while later four are gram negative bacteria. Antibacterial activities of all samples were screened by the agar well diffusion method [27, 28]. Compounds 6d, 6e and 6a were most potent and comparable to activities of standard antibiotic chloramphenicol against Bacillus megaterium, Micrococcus luteus Staphylococcus, Proteus vulgaris and Enterobacter. Weak activity was observed with the other compound 6b and 6c.

Table 7: Anti-bacterial activity of Pyrazoline derivatives.

Anti-microbial Activity of Synthetic Compound (In mm)								
Organisms								
Compounds	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Chloramphenicol	+++	+++	+++	+++	+++	++	++	++
6a	-	-	-	-	-	+++	++	-
6b	++	-	-	-	-	-	-	-
6c	-	-	++	-	-	++	-	-
6d	+++	-	+++	-	-	-	-	-
6e	+++	-	-	++	-	-	+++	-
6f	++	-	++	-	-	-	-	-
6g	-	-	-	-	-	-	-	-
A: Bacillus megaterium, B: Bacillus subtilus, C: Micrococcus luteus, D: Staphylococcus aureus, E: Eschericha coli, F: Enterobacter, G: Proteus vulgaris, H: Pseudomonas aeruginosa								
+ : <6 mm (poor), ++ : <12 mm (good), +++ : <18 mm (v.good), - : Without activity								

Antifungal activity

All the synthesized compounds were also screened for their in vitro anti-fungal activity against Mucor, A. niger and Penicillium strains. The zone of inhibition was measured in millimeters. Antifungal activities of all compounds were screened by the turbidimetry method [29]. Activity of extract was compared with standard antibiotics fluconazole fungi. DMSO was used as solvent. All compounds are active against Mucor, A. niger and penicillium. Compounds 6d, 6a, 6e provided the best antifungal activity and compared well with the activity of fluconazole. The compounds 6f and 6c also possess promising antifungal activity.

Table 8: Anti-Fungal activity of Pyrazoline derivatives.

Antifungal Activity of Synthetic compound (In mm)			
Compounds	**Organisms**		
	A	**B**	**C**
Fluconazole	++	+++	+++
6a	++	+++	+++
6b	+	++	+
6c	+	++	++
6d	+++	+++	+++
6e	++	++	+++
6f	+	+++	++
6g	+	+	+
A: Mucor, B: Penicillium, C:Aspergillus			
+ : <25 mm (poor), ++ : <50 mm (good), +++ : <75 mm (very good)			

Results and Discussions

Chemistry

Factors Such as The structure and position of the substituents effects the rate of reaction. The generally accepted interpretation of this reaction, involves the initial formation of an hydrazone with subsequent nucleophilic attack of nitrogen upon the carbon-carbon double bond at β position. Hence The Electropositive nature of β carbon may control the over all rate of the reaction. Electron withdrawing groups like Nitro group Significantly Increase the Positive Character of beta carbon lead to faster reaction while Electron donating groups like alkyl and alkoxy groups contributed for Slower reaction.

Structures of Compounds 6a-6g were confirmed by IR, ^{1}H NMR Spectroscopic Technique. All of the Pyrazoline possesses Similar basic skeletal structure.

Characterization

The IR spectrum of the title Compounds 6(a-g) has given stretching vibration at 3420 cm^{-1} due to the stretching vibration corresponding to N-H Stretching vibrations. 3100cm^{-1}, due to the stretching vibration corresponding to Ar-H Stretching vibrations. The absorption peak at 2935 cm^{-1} is due to the stretching vibration corresponding to the SP^3 C-H (methyl gp).The strong Intensity absorption at 1350 & 1530 cm^{-1} is due to the stretching vibration of -N-O Stretching in Nitro group, 1360 cm^{-1} is due to the stretching vibration of C-F bond. 760 cm^{-1} is due to the stretching vibration of C-Cl bond. 560 cm^{-1} is due to the stretching vibration of C-Br bond. The weak Intensity absorption at 1620 cm^{-1} corresponds to a C=N Stretching vibration.1150cm^{-1} corresponding to C-O Stretching.

It has been observed from chemical structure of compound 8(a-g) that different pair of protons. The protons of Methyl group which is attached to benzene ring appeared as a singlet at δ =2.3 ppm, the protons of Methoxy group appeared as a Singlet at δ =3.8 ppm, the protons attached benzene ring appeared between δ =7.2-8.3 ppm respectively.

The chemical shifts of the final compound carbon vary from δ = 165 to 23 ppm. The carbon nucleus under the influence of a strong electronegative environment appeared down field, the carbon chemical shift of the methyl group at δ= 23 ppm. The carbon chemical shift of the Methoxy group at δ= 55 ppm.

Readily available starting materials and Simple Synthesizing procedures make this method very attractive and convenient for the synthesis of Pyrazoline derivatives. Formation of products was confirmed by recording their Elemental analysis, ^{1}H NMR, ^{13}C, FT-IR, mass spectra. The Elemental analysis data showed good agreement between the experimentally determined values and the theoretically calculated values with in ±0.4%.

Anti -microbial screening

The results of Anti -microbial studies of newly synthesized compounds reveal that the compounds possess significant Anti -microbial activities. The results of these studies are given in Table 7 & 8. From Anti -Microbial screening results, it has been observed that compounds 6d possess good activity.

Conclusions

We have synthesized a series of new Pyrazoline derivatives 6(a-g) containing bioactive hetero aryl pharmacophores such as phenothiazine ring using convenient method.

In conclusion, the present investigation reports the synthesis of Pyrazoline derivatives and the evaluation of their anti-bacterial & Anti-Fungal Activity. All compounds were screened for their in vitro antifungal against mucor, aspergillus and pecillium and in vitro antibacterial against Bacillus megaterium, Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Eschericha coli, Enterobacter, Proteus vulgaris and Pseudomonas aeruginosastrains. Zone of inhibition were measured in millimeters. The antifungal activities of the tested compounds were compared with standard drug Fluconazole. DMF was used as solvent. Compound 6d, 6a and 6e found most active against all three fungi compared to standard drug. Chloramphenicol was used as standard drugs for antibacterial activity. The compounds 6d, 6e and 6a showed significant activity against Bacillus megaterium, Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Eschericha coli, Enterobacter, Proteus vulgaris and Pseudomonas aeruginosa strains.

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