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SYNTHESIS, CHARACTERISATION AND ANTI-MICROBIAL ACTIVITY OF SOME NOVEL PYRAZOLINE DERIVATIVES HAVING THIENO [2, 3-*D*] PYRIMIDINE AS A CORE UNIT

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Abstract: A new series of 6-(3-p-Substituted-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidinederivatives (5a-j) were synthesized by reacting (E)-3-(thieno[2,3-*d*]pyrimidin-6-yl)-1-p-substituted prop-2-en-1-one(4a-4j) with hydrazine hydrate respectively. All these compounds were characterized by means of their IR, ^{1}H NMR, mass spectral data and microanalysis. All the synthesized products were evaluated for their antimicrobial activity. All the compounds exhibited significant to moderate antimicrobial activity. Compounds 5e, 5d, 5i and 5g demonstrated good antimicrobial activity against all the tested microbial stains compared with Standard Drugs.

Keywords: chalcones, Thieno-pyrimidines, 2-Pyrazolines, Antimicrobial 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 [**I**]. **Pyrazoline** is a mono cyclic heterocyclic compound consists of a five membered ring with two nitrogen atoms at 1 & 2 Position [**Fig.1** Structure of Pyrazoline]. 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 **[II-IV].** The past studies of substituted pyrazolines revealed that they exhibit antibacterial **[V]**, analgesic **[VI]**, anti-inflammatory **[VII],** anti-viral **[VIII],** anti-fungal **[IX],** anti-arthritic **[X],** cerebroprotective effect **[XI],** and

anti-depressant **[XII]** properties. There are several substituted pyrazolines having bleaching property or act as luminescent and fluorescent agents **[XIII].** They are also useful as biodegradable agrochemicals **[XIV].**

A variety of substituted pyrazolines [**XV**] 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 [**XVI**], anti-diuretic [**XVII**], anti-analgesic [**XVIII**], anti-helimentic [**XIX**], anti-hypolipaemic [**XX**], anti-malarial [**XXI**], and anti-depressant [**XXII**] activities.

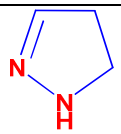


Fig.1 Structure of Pyrazolinepyrazoline

Chalcones constitute an important class of natural products and some of them possess a wide range of pharmacological activities such as anticancer, anti-tubercular, antiviral [**XXIII**]. Recent studies on biological evaluation of Chalcones revealed some to be antibacterial, antifungal, Anti-inflammatory, anti hyperglycaemic [**XXIV**], and anti-malarial agents [**XXV**].

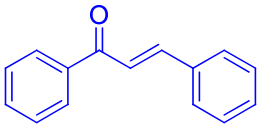
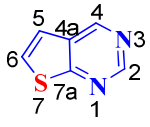


Fig.2 General structure of Chalcones

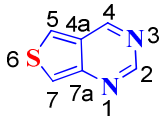
The chalcones are unsaturated ketones containing the reactive keto ethylene group

Thieno Pyrimidine is a bi cyclic heterocyclic compound consists of a five membered thiophene ring is fused to a six membered hetero cyclic ring with two nitrogen atoms. The fusion may occur in three different orientations that results in three important types of thienopyrimidines namely; **Thieno[2,3-*d*]Pyrimidine (a)**, thieno[3,2-*d*]Pyrimidine (b) and thieno[3,4-*d*]Pyrimidine (c).Most of the isomeric thienopyrimidines occur as colored amorphous form, some exists as crystalline form.

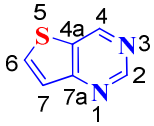
Synthetic approaches for the construction of a number of thieno Pyrimidines are well established. There exists three possible types of fusion of thiophene to Pyrimidine ring results in corresponding isomeric thienopyrimidines namely**;[Fig.3]** thieno[2,3-*d*]pyrimidines (a), thieno[3,4-*d*]pyrimidines (b) and thieno[3,2-*d*]pyrimidines (c).



thieno[2,3-d]pyrimidine (a)



thieno[3,4-d]pyrimidine (b)



thieno[3,2-d]pyrimidine (c)

Fig: 3 Structures of different isomers of Thieno Pyrimidine

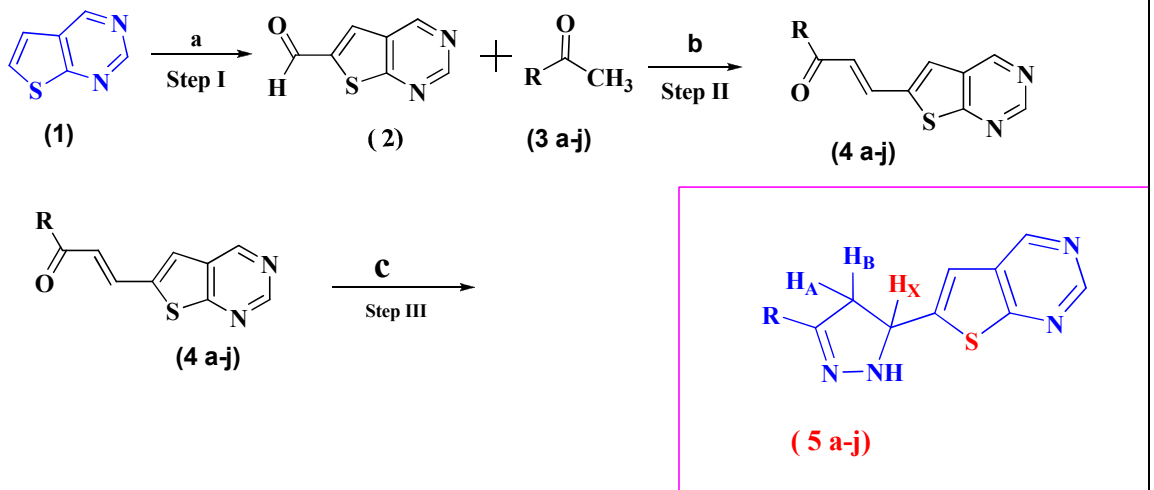
Thiophene containing compounds are well known to exhibit various biological effects. Heterocycles containing the thienopyrimidine moiety are of interest because of their interesting pharmacological and biological activities [**XXVI– XXVIII**]. They bear structural analogy and isoelectronic relation to purine and several substituted thieno [2, 3-*d*] Pyrimidine derivatives shown to exhibit prominent and versatile biological activities [**XXIX, XXX**]. Over the last two decades, many thieno-pyrimidines have been found to exhibit a variety of pronounced activities. Many of their derivatives have been synthesized as potential anticancer [**XXXI**], analgesic [**XXXII**], antimicrobial [**XXXIII, XXXIV**] and antiviral agents [**XXXV**]. Encouraged by these facts and in continuation with the wok related to the synthesis, spectral studies and biological properties of pyrazolines, here it is reported the synthesis of some novel pyrazolines having Thieno [2, 3-*d*] Pyrimidine core unit, then their antibacterial and anti-Fungal activities were investigated. **MATERIALS AND METHODS**

In this Investigation chemicals were purchased from local dealer with S.D fine & Avra labs make was used. Chemicals were 99 % pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of thieno [2, 3-*d*] Pyrimidine derivatives. Stirring and reflux method were used for synthesis of Thieno [2, 3-*d*] Pyrimidine derivatives 6 (a-j) respectively.

The synthetic route was depicted in scheme **I.**

The title compounds 5(a-j) were synthesized in three sequential steps using different reagents and reaction conditions, the 5(a-j) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) data.

Synthetic Scheme

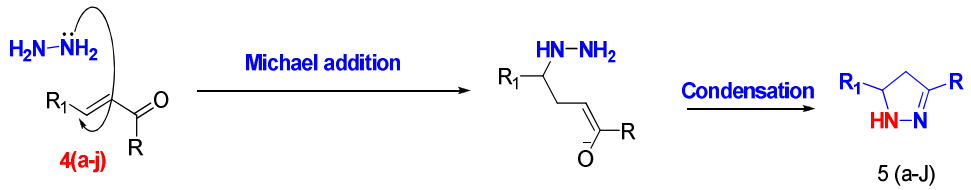


R = -Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 tri fluoro methoxy phenyl, -4 Tri fluoro phenyl, -4 Nitro phenyl, - pyrazin-2-yl, - pyridin-4-yl, Thiophene 2-yl, Furan-2-yl acetyl groups.

Scheme: I Synthetic path way of preparation of novel pyrazolines containing Thieno [2, 3-*d*] Pyrimidine Nucleus (6 a-j).

Reagents and Reaction conditions: (a) *DMF,POCl$_3$,80^{O}C,4hrs* (b) *NaOH,Ethanol, RT, 24 hrs* (c) *Hydrazine hydrate (***NH_2- $NH_2.H_2O$***) , Methanol, Reflux, 4hrs.*

Possible Mechanism For Formation of Pyrazoline Heterocyclic ring 5(a-j) Formation :



R_1 = Thieno[2,3-*d*]pyrimidine 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. N, N Di Methyl Formamide (DMF) was distilled from CaH_2 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 ^1H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane 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 (^1H NMR and ^{13}C NMR) were recorded using tetra methyl silane (TMS) in the solvent of $CDCl_3$-d_1 or DMSO-d_6 as the internal standard (^1H 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).

The antimicrobial tests were carried out at the Bio-Technology Department, Faculty of Sciences, Sri Krishnadevaraya University. ChemDrawUltra-12.0 has been used for the nomenclature of the prepared compounds.

Synthesis:

General procedure for synthesis of thieno [2, 3-d] pyrimidine-6-carbaldehyde [Compound 2]:

thieno [2,3-*d*] Pyrimidine (1) (10 g, 0.0735 mol) was dissolved in dry DMF(100 mL),under anhydrous condition, it was cooled to 0^OC, $POCl_3$ (15 mL) was added drop wise for 30 min. and stirring continued for 4 h at 80^oC After completion of reaction (TLC), The reaction mass was poured over crushed ice, basified with NaOH , Extracted with chloroform and dried over anhydrous Na_2SO_4 . Organic layer was concentrated under reduced pressure and purified through silica gel column (Neutral Alumina) using Chloroform as eluting solvent to yield product (2) [yield 60%, 7.2g].

IR (KBr, cm^{-1}): 3110 cm^{-1} (Ar C-H stret), 2720 (C-H Stretch), 1725 cm^{-1} (C=O Stretch),1550 cm^{-1} (C=C Stret), Wave numbers respectively.

^1H NMR (400 MHz; $CDCl_3$): δH 8.25 (S, 1H, Ar-H), 8.83 (S, 1H, Ar-H), 9.45 (S, 1H, Ar-H), 10.05 (S,

-**H**-C=O).

^{13}C NMR (100 MHz; $CDCl_3$): δC 130, 135, 145, 149, 158, 195.

MS (70 eV): m/z = $165(M+H)^+$.

General procedure for synthesis of (E)-1-phenyl-3-(thieno [2, 3-d]pyrimidin-6-yl)prop-2-en-1-one (4a), (E)-3-(thieno[2,3-d]pyrimidin-6-yl)-1-p-tolylprop-2-en-1-one(4b), (E)-1-(4-methoxyphenyl)-3-(thieno[2,3-d]pyrimidin-6-yl)prop-2-en-1-one (4c), (E)-3-(thieno[2,3-d]pyrimidin-6-yl)-1-(4-(trifluoromethoxy)phenyl)prop-2-en-1-one (4d), (E)-3-(thieno[2,3-d]pyrimidin-6-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (4e), (E)-1-(4-nitrophenyl)-3-(thieno[2,3-d]pyrimidin-6-yl)prop-2-en-1-one (4f), (E)-1-(pyrazin-2-yl)-3-(thieno[2,3-d]pyrimidin-6-yl)prop-2-en-1-one (4g), (E)-1-(pyridin-4-yl)-3-(thieno[2,3-d]pyrimidin-6-yl)prop-2-en-1-one (4h),(E)-3-(thieno[2,3-d]pyrimidin-6-yl)-1-(thiophen-2-yl)prop-2-en-1-one(4i), (E)-1-(furan-2-yl)-3-(thieno[2,3-d]pyrimidin-6-yl)prop-2-en-1-one (4j):

Various acetyl derivatives (3 a-j) (10 m.mol) were dissolved in ethanol, 10 mL 20% NaOH Solution was added to it. and stirred for 10 min at RT. Then thieno[2,3-*d*]pyrimidine-6-carbaldehyde (2) was added and stirring continued for 24h at Room temperature, after completion of reaction (TLC), reaction mixture was poured over crushed ice and stirred. The precipitate obtained was filtered and recrystallised by using Ethanol to obtain the chalcone derivatives (4a-j).

(E)-1-phenyl-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4a):

Yield: 85% (yellow colour solid);

IR (KBr, cm^{-1}): 3140(-Ar CH), 1652 (C=O Stretching), 1620(C=C Stretching), 675(C-S-C).

^{1}H NMR (400 MHz; $CDCl_3$): δH 9.44 (S, 1H, Ar-H), 8.85 (S, 1H, - Ar-H), 8.32(S,1H), 7.56 (d, 1H, CO-CH=, J=15.4Hz), 7.95 (d, 1H, β C-H, J=15.4Hz), 7.62-7.95(5H,m).

^{13}C NMR (100 MHz; $CDCl_3$): δC 128.92, 124.03, 128.11, 151.67, 154.75, 159.62, 195.

MS (70 ev): m/z = $266(M+H)^+$.

(E)-3-(thieno [2, 3-*d*] pyrimidin-6-yl)-1-p-tolylprop-2-en-1-one (4b):

Yield: 86% (light yellow colour solid);

IR (KBr, cm^{-1}): 3120(-Ar CH), 2970(SP^3 CH), 1675 (C=O Stretching), 1630(C=C Stretching), 668(C-S-C).

^{1}H NMR (400 MHz; $CDCl_3$): δH 9.4 (S, 1H, Ar-H), 8.8 (S, 1H, - Ar-H), 8.23(S,1H), 7.56 (d, 1H, CO-CH=, J=15.6Hz), 7.95 (d, 1H, β C-H, J=15.6Hz), 7.98(2H,d, J=7.2Hz), 7.4(2H,d, J=7.2Hz), 2.3(3H,S).

^{13}C NMR (100 MHz; $CDCl_3$): δC 23, 125, 128.92, 124.03,135, 151.67, 154.75, 159.62, 190.

MS (70 ev): m/z = $281(M+H)^+$.

(E)-1-(4-methoxyphenyl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4c):

Yield: 90% (yellow colour solid);

IR (KBr, cm^{-1}): 3120(-Ar CH), 2970(SP^3 CH), 1655 (C=O Stretching), 1630(C=C Stretching), 1160(C-O-C Stretching), 668(C-S-C).

^{1}H NMR (400 MHz; $CDCl_3$): δH 9.45 (S, 1H, Ar-H), 8.85 (S, 1H, - Ar-H), 8.23(S, 1H), 7.56 (d, 1H, CO-CH=, J=15.3Hz), 7.95 (d, 1H, β C-H, J=15.3Hz), 8.25(2H, d, J=7.4Hz), 7.2(2H, d, J=7.4Hz), 3.89(3H, S).

^{13}C NMR (100 MHz; $CDCl_3$): δC 56, 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 185.

MS (70 ev): m/z = $297(M+H)^+$.

(E)-3-(thieno [2, 3-*d*] pyrimidin-6-yl)-1-(4-(tri fluoro methoxy) phenyl)prop-2-en-1-one (4d):

Yield: 90% (yellow colour solid);

IR (KBr, cm^{-1}): 3110(-Ar CH), 1640 (C=O Stretching), 1625(C=C Stretching), 1340(C-F), 1160(C-O-C), 675(C-S-C).

^{1}H NMR (400 MHz; $CDCl_3$): δH 9.44 (S, 1H, Ar-H), 8.83 (S, 1H, - Ar-H), 8.23(S, 1H), 7.56 (d, 1H, CO-CH=, J=15.6Hz), 7.95 (d, 1H, β C-H, J=15.6Hz), 8.25(2H, d, J=7.2Hz), 7.2(2H,d, J=7.2Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 190.2.
MS (70 ev): m/z = 351$(M+H)^+$.

(E)-3-(thieno [2, 3-*d*] pyrimidin-6-yl)-1-(4-(trifluoro methyl) phenyl) prop-2-en-1-one (4e):
Yield: 90% (yellow colour solid);
IR (KBr, cm^{-1}): 3130(Ar CH), 1665 (C=O Stretching), 1640(C=C Stretching), 1360(C-F), 685(C-S-C).
^{1}H NMR (400 MHz; $CDCl_3$): δH 9.44 (S, 1H, Ar-H), 8.8 (S, 1H, - Ar-H), 8.23(S,1H), 7.55 (d, 1H, CO-CH=, J=15.6Hz), 7.90 (d, 1H, β C-H, J=15.6Hz), 8.1(2H,d, J=7.3Hz), 7.8(2H,d, J=7.3Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 189.
MS (70 ev): m/z = 335$(M+H)^+$.

(E)-1-(4-nitrophenyl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4f):
Yield: 80% (yellow colour solid);
IR (KBr, cm^{-1}): 3110(Ar CH), 1655 (C=O Stretching), 1646(C=C Stretching), 1336 & 1550 (N-O Symmetric & Asymmetric stretching in Nitro Group), 680(C-S-C).
^{1}H NMR (400 MHz; $CDCl_3$): δH 9.45 (S, 1H, Ar-H), 8.83 (S, 1H, - Ar-H), 8.23(S,1H), 7.55 (d, 1H, CO-CH=, J=15.4Hz), 7.95 (d, 1H, β C-H, J=15.4Hz), 8.2(2H,d, J=7.4Hz), 8.5(2H,d, J=7.4Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 189.
MS (70 ev): m/z = 310$(M-H)^+$.

(E)-1-(pyrazin-2-yl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4g):
Yield: 80% (yellow colour solid);
IR (KBr, cm^{-1}): 3100(Ar CH), 1670 (C=O Stretching), 1655(C=C Stretching), 1470(C=N), 685(C-S-C).
^{1}H NMR (400 MHz; $CDCl_3$): δH 9.32 (d, 1H, J=2.6 HZ, Ar-H), 8.94 (S, 1H, J=2.6 HZ, - Ar-H),
8.35(S, 1H), 6.75 (d, 1H, J=15.6 Hz, CO-CH=), 7.65 (d, 1H, J=15.6 Hz, β C-H), 8.84(1H, J=7.6 Hz, d), 8.90(1H, J=7.6 Hz,d), 9.45(1H,S).
^{13}C NMR (100 MHz; $CDCl_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 195.
MS (70 ev): m/z = 267$(M-H)^+$.

(E)-1-(pyridin-4-yl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4h):
Yield: 82% (yellow colour solid);
IR (KBr, cm^{-1}): 3100(Ar CH), 1670 (C=O Stretching), 1655(C=C Stretching), 1460(C=N), 685(C-S-C).
^{1}H NMR (400 MHz; $CDCl_3$): δH 9.34(S, 1H, Ar-H), 8.82 (S, 1H, - Ar-H), 8.25(S, 1H), 6.75 (d, 1H, CO-CH=, J=15.4Hz), 7.55 (d, 1H, β C-H, J=15.4Hz), 8.90(1H, d, J=7.2Hz), 8.12(1H, d, J=7.2Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 195.
MS (70 ev): m/z = 266$(M-H)^+$.

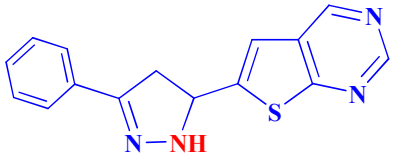
(E)-3-(thieno [2, 3-*d*] pyrimidin-6-yl)-1-(thiophen-2-yl)prop-2-en-1-one (4i):

Yield: 80% (yellow colour solid);
IR (KBr, cm^{-1}): 3100(Ar CH), 1670 (C=O Stretching), 1655(C=C Stretching), 1470(C=N), 685(C-S-C).
^{1}H NMR (400 MHz; CDCl$_3$): δH 9.35 (S, 1H, Ar-H), 8.83 (S, 1H, - Ar-H), 8.33(S, 1H), 6.86 (d, 1H, CO-CH=, J=15.4Hz), 7.68 (d, 1H, β C-H, J=15.4Hz), 8.12(1H, d, J=6.8Hz), 8.26(1H, d J=6.8Hz), 7.42(1H, t, J=6.8Hz).
^{13}C NMR (100 MHz; CDCl$_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 195.
MS (70 ev): m/z = 273.10(M+H)$^{+}$.

(E)-1-(furan-2-yl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4j):
Yield: 82% (yellow colour solid);
IR (KBr, cm^{-1}): 3100(Ar CH), 1670 (C=O Stretching), 1655(C=C Stretching), 1460(C=N), 685(C-S-C).
^{1}H NMR (400 MHz; CDCl$_3$): δH 9.45 (S, 1H, Ar-H), 8.83 (S, 1H, - Ar-H), 8. 25(S, 1H), 6.75 (d, 1H, CO-CH=, J=15.6Hz), 7.65 (d, 1H, β C-H, J=15.6Hz), 8.12(1H, d, J=7.4Hz), 7.92(1H, t, J=7.4Hz), 8.72(1H, d, J=7.4Hz).
^{13}C NMR (100 MHz; CDCl$_3$): δC 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 195.
MS (70 ev): m/z = 255(M-H)$^{+}$.

General procedure for the synthesis of 2-pyrazoline (5a-j)
A mixture of chalcones 4a-4j (0.01moles) and hydrazine hydrate (0.02 moles) in 50 mL methanol was reflux for 2 h, excess methanol was distilled and the resulting solution was kept overnight. Crystalline solid filtered and crystallized from ethanol.

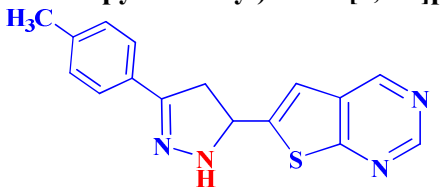
6-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5a)



structure of 6-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5a)

Yield: 78% (white colour solid);
m.p.126^{O}C.
IR (KBr, cm^{-1}): 3140(-Ar CH), 1645 (C=N of pyrazoline ring), 1540 (C=C),.
^{1}H NMR (400 MHz; DMSO-d$_6$): δH 9.45 (S, 1H, Ar-H), 8.85 (S, 1H, - Ar-H), 6.82(S, 1H), 7.65-7.95(5H, m), 3.96 (dd, 1H, J=10.8 Hz, HA), 3.65 (dd, 1H, J=11.2 Hz, HB), 3.92 (dd, 1H, J=11.2, Hx),7.4(1H,bs).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 43, 52,125,130,143,147.73, 150, 152.5, 157.1.
MS (70 ev): m/z = 281(M+H)$^{+}$.

6-(3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5b)

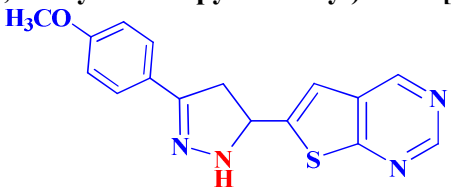


structure of 6-(3-*p*-tolyl-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5b)

Yield: 82% (white colour crystals);
m.p.131^{O}C.
IR (KBr, cm^{-1}): 3110(-Ar CH), 2990(SP^3 CH), 1655 (C=N of pyrazoline ring), 1560 (C=C).

^{1}H NMR (400 MHz; DMSO-d_6): δH 2.35(3H,S),9.35 (S, 1H, Ar-H), 8.85 (S, 1H, - Ar-H), 6.72(S, 1H), 7.33(2H, d, J=7.3Hz), 7.83(2H, d, J=7.3Hz), 3.95 (dd, 1H, J=10.6 Hz, HA), 3.65 (dd, 1H, J=11.4 Hz, HB), 3.95 (dd, 1H, J=12.2, Hx), 7.93(1H,bs).
^{13}C NMR (100 MHz; DMSO-d_6): δC 23, 43, 53,125,130,143,147.73, 150, 158.5.
MS (70 ev): m/z = 295(M+H)$^{+}$.

6-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5c):

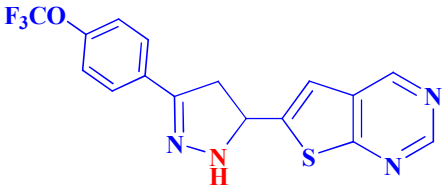


structure of 6-(3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5c)

Yield: 85% (off white colour solid);
m.p.196^{O}C.
IR (KBr, cm^{-1}): 3120(-Ar CH), 1640 (C=N of pyrazoline ring), 1165(-OCH_3), 1540 (C=C), 678(C-S-C).
^{1}H NMR (400 MHz; DMSO-d_6): δH 3.89(3H,S), 9.35 (S, 1H, Ar-H), 8.85 (S, 1H, - Ar-H), 6.72(S, 1H), 7.13(2H, d, J=7.1Hz), 7.93(2H, d, J=7.1Hz), 3.90 (1H, dd, J=11.4 Hz, HA), 3.65 (dd, 1H, J=11.4 Hz, HB), 3.92 (dd, 1H, J=12.2Hz, Hx) , 7.53(1H,bs).

^{13}C NMR (100 MHz; DMSO-d_6): δC 43, 52, 58, 115,120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 163.1.
MS (70 ev): m/z = 311(M+H)$^{+}$.

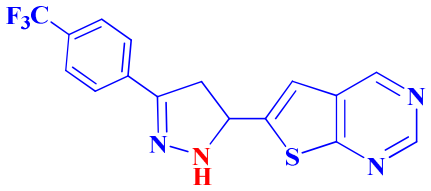
6-(3-(4-(trifluoromethoxy)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5d):



structure of 6-(3-(4-(trifluoromethoxy)phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5d)

Yield: 84% (light yellow colour solid);
m.p.166^{O}C.
IR (KBr, cm^{-1}): 3110(-Ar CH), 1648 (C=N of pyrazoline ring), 1155 (-OCH_3), 1545 (C=C), 1340(C-F), 675(C-S-C).
^{1}H NMR (400 MHz; DMSO-d_6): δH 9.28 (S, 1H, Ar-H), 8.79 (S, 1H, - Ar-H), 6.62(S, 1H), 7.18(2H, d, J=7.1Hz), 7.95(2H, d, J=7.1Hz), 3.90 (1H, dd, J=11.5 Hz, HA), 3.65 (dd, 1H, J=11.5 Hz, HB), 3.89 (dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 52, 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 156.62, 159.23.
MS (70 ev): m/z = 365. 15 (M+H)$^{+}$.

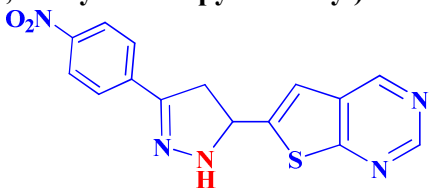
6-(3-(4-(trifluoromethyl) phenyl)-4, 5-dihydro-1H-pyrazol-5-yl) thieno [2, 3-*d*] pyrimidine (5e):



structure of 6-(3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine(5e)

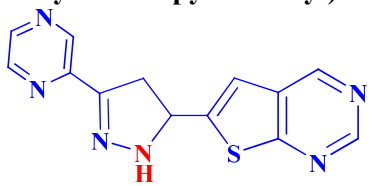
Yield: 86% (yellow colour solid);
m.p.198^{O}C.
IR (KBr, cm^{-1}): 3130(Ar CH), 1644 (C=N of pyrazoline ring), 1540 (C=C), 1380(C-F), 685(C-S-C).
^{1}H NMR (400 MHz; DMSO-d$_6$): δH 9.25 (S, 1H, Ar-H), 8.76 (S, 1H, - Ar-H), 6.72(S, 1H), 7.88(2H, d, J=7.1Hz), 8.15(2H, d, J=7.1Hz), 3.90 (1H, dd, J=11.7 Hz, HA), 3.65 (dd, 1H, J=11.7 Hz, HB), 3.85 (dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 46, 52, 120,125, 128.72, 124.13,135, 149,151.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 349.08(M+H)$^{+}$.

6-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5f):



structure of 6-(3-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5f)
Yield: 84% (yellow colour solid);
m.p.208^{O}C.
IR (KBr, cm^{-1}): 3110(Ar CH), 1650.5 (C=N of pyrazoline ring), 1545 (C=C), 1336 & 1550 (N-O Symmetric & Asymmetric stretching in Nitro Group),
680(C-S-C).
^{1}H NMR (400 MHz; DMSO-d$_6$): δH 9.25 (S, 1H, Ar-H), 8.75 (S, 1H, - Ar-H), 6.72(S, 1H), 8.35(2H, d, J=7.1Hz), 8.05(2H, d, J=7.1Hz), 3.90 (1H, dd, J=10.7 Hz, HA), 3.65 (dd, 1H, J=10.7 Hz, HB), 3.95(dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 46, 51, 125, 128.72, 135, 143,145, 150.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 324.347(M-H)$^{+}$.

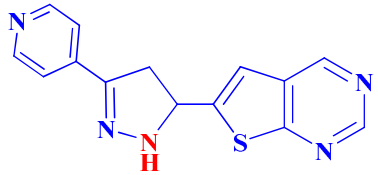
6-(3-(pyrazin-2-yl)-4, 5-dihydro-1H-pyrazol-5-yl) thieno [2, 3-*d*] pyrimidine (5g):



structure of 6-(3-(pyrazin-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5g)
Yield: 80.5% (off white colour solid);
m.p.238^{O}C.
IR (KBr, cm^{-1}): 3100(Ar CH), 1655 (C=N of pyrazoline ring), 1540 (C=C), 685(C-S-C).

^{1}H NMR (400 MHz; DMSO-d_6): δH 9.25 (S, 1H, Ar-H), 8.75 (S, 1H, - Ar-H), 6.72(S, 1H), 9.55(1H, S), 8.75(1H, d, J=7.1Hz), 8.9(1H,d,J=7.1Hz), 2.60 (1H, dd, J=10.4 Hz, HA), 2.25 (dd, 1H, J=10.4 Hz, HB), 3.93(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 46, 51.2, 125, 128.72, 135, 143,145, 150.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 281.325(M-H)$^{+}$.

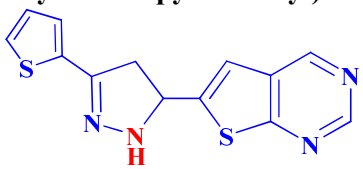
6-(3-(pyridin-4-yl)-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5h):



structure of 6-(3-(pyridin-4-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5h)

Yield: 75% (yellow colour solid);
m.p.269^{O}C.
IR (KBr, cm^{-1}): 3120(Ar CH), 1652 (C=N of pyrazoline ring), 1560 (C=C), 685(C-S-C).
^{1}H NMR (400 MHz; DMSO-d_6): δH 9.25 (S, 1H, Ar-H), 8.78 (S, 1H, - Ar-H), 6.62(S, 1H), 9.55(1H, S), 8.75(2H, d, J=7.1Hz), 7.49(2H,d,J=7.1Hz), 2.60 (1H, dd, J=10.4 Hz, HA), 2.25 (dd, 1H, J=10.4 Hz, HB), 3.90(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 54.2, 125, 128.72, 138, 144,148, 156.62, 159.23.
MS (70 ev): m/z = 281(M-H)$^{+}$.

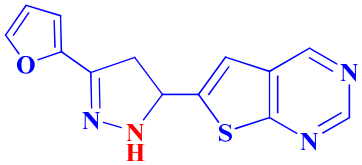
6-(3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5i) :



structure of 6-(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine(5i)

Yield: 78% (pale yellow colour solid);
m.p.227^{O}C.
IR (KBr, cm^{-1}): 3100(Ar CH), 1655 (C=N of pyrazoline ring), 1560 (C=C), 685(C-S-C).
^{1}H NMR (400 MHz; DMSO-d_6): δH 9.35(S, 1H, Ar-H), 8.85(S, 1H, - Ar-H), 6.60(S, 1H), 7.82(1H, d, J=6.9Hz), 7.35(1H, t, J=6.9 Hz), 7.69(1H,d,J=6.9Hz), 2.65 (1H, dd, J=10.4 Hz, HA), 2.25 (dd, 1H, J=10.4 Hz, HB), 3.84(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 53, 125, 128.72,143, 147,149,157.
MS (70 ev): m/z = 286.36(M-H)$^{+}$.

6-(3-(furan-2-yl)-4, 5-dihydro-1H-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine (5j):



structure of 6-(3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)thieno[2,3-*d*]pyrimidine(5j)

Yield: 80% (off white colour solid);
m.p. 255^{O}C.
IR (KBr, cm^{-1}): 3110(Ar CH), 1665 (C=N of pyrazoline ring), 1565 (C=C), 680(C-S-C).

^{1}H NMR (400 MHz; DMSO-d_6): δH 9.25(S, 1H, Ar-H), 8.75(S, 1H, - Ar-H), 6.60(S, 1H), 7.72(1H, d, J=6.9Hz), 6.55(1H, t, J=6.9 Hz), 6.93(1H,d,J=6.9Hz), 2.65 (1H, dd, J=10.4 Hz, HA), 2.25 (dd, 1H, J=10.4 Hz, HB), 3.87(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 53, 125, 128.72,143, 147,149,157.
MS (70 ev): m/z = 269.342$(M-H)^+$.

Biological Activity:

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli (clinical isolate) bacterial strains by disc diffusion method [**XXXVI, XXXVII**]. A standard inoculums (1-2×107 c.f.u./ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from what man no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. **Amoxicillin** (30 µg) was used as positive control and the disk poured in DMSO was used as negative control and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/ml. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values are given in (Table 1). The order of activity was **5e>5d>5i>5g>5f>5h>5j>5b>5c>5a.**

Table 1: Anti-bacterial activity of Novel Isoxazoline derivatives 5(a-j):

Zone of inhibition measure in mm								
Synthesised Compounds	**Gram positive**				**Gram negative**			
	Bacillus sub tilis		**Staphylocouccus aurous**		**Klebsiella pneumonia**		**Escherichia coli**	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
5a	6	3	7.5	5	8	6	9.5	6
5b	7.5	3.5	8	7	9.5	7	10.5	7.5
5c	7	4.5	7	4.5	8.5	6.5	9	7
5d	12.5	10	14.5	10.5	15	13.5	16.5	12.5
5e	13	10.5	15	11.5	16.5	14	17	13
5f	10	8	11.1	9.5	12	11	13.5	11
5g	11	9.5	11.5	8.5	12.5	12	13	11.5
5h	9.5	7	9.5	7.5	12	10	12.5	10.5
5i	11.5	9	12.5	11	14.5	11.5	15.5	12
5j	8.5	6.5	9.0	6.5	10.15	8	11	8
Amoxicillin	15.5	12.8	17.6	13	18	14.5	19.5	15.6
Control (**DMSO**)	---	---	----	-----	----	-----	-----	-----

Anti-fungal studies

The newly prepared compounds were screened for their antifungal activity against **Candida albicans** and **Aspergillus flavus** in DMSO by agar diffusion method [**XXXVIII**].

Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting p^H 5.7. Normal saline was used to make suspension of corresponding species. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The fungal activity of each compound was compared with **Flucanazole** as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values are given in (**Table 2**).

Table 2: Anti-fungal activity of Novel Thieno [2, 3-*d*] Pyrimidine derivatives 7(a-j):

Zone of inhibition measure in mm				
Synthesised Compounds	**Candida albicans**		**Aspergillus flavus**	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
5a	6.5	4.5	7	4
5b	8	5.5	7	3.5
5c	7.5	3.5	8	7
5d	14.5	12	12.5	9.5
5e	17.5	12.5	16	12
5f	10	8	11.1	9.5
5g	11	9.5	11.5	8.5
5h	9.5	7	9.5	7.5
5i	13	11.5	10.5	8
5j	8.5	6.5	9.0	6.5
Flucanazole	21	16	18.5	14
Control (**DMSO**)	---	---	---	---

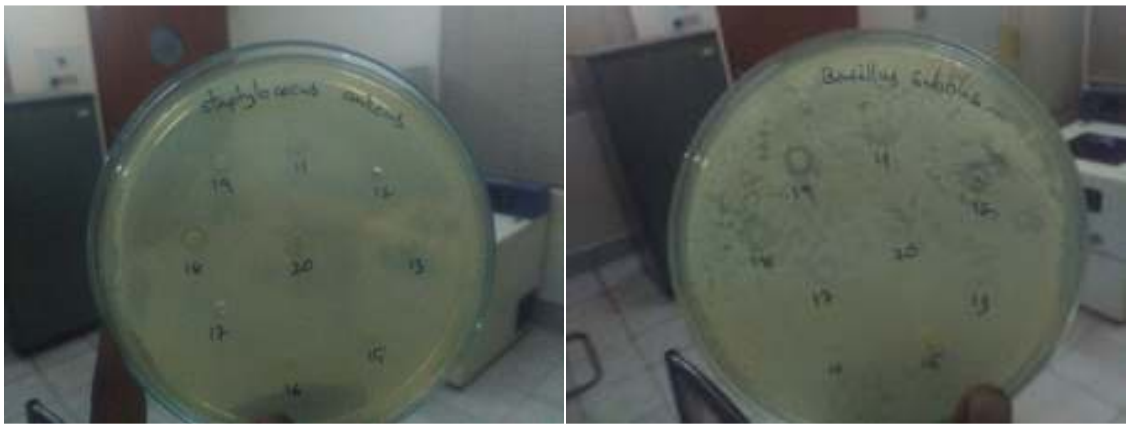


Fig.4 Anti-bacterial activity Images of Novel Pyrazoline derivatives (5a-5j).

Results and Discussion:

Chemistry:

The Title Compounds Novel Pyrazolines containing thieno [2, 3-*d*] Pyrimidine based derivatives 5(a-j) were synthesized in good yields (scheme-I). All these compounds were

tested for Anti-microbial activity showed considerable activity when compared to the standard drug.

In the present communication thieno [2, 3-*d*] pyrimidine-6-carbaldehyde (2) was synthesised from thieno [2, 3-*d*] pyrimidine (1) According to the reported procedure [**XXXIX**]. Various chalcone derivatives 4(a-j) was from synthesised thieno [2, 3-*d*] pyrimidine-6-carbaldehyde (2) & different substituted acetyl groups According to the reported procedure [**XL**], these are further reacted with hydrazine hydrate in methanol to get target novel isoxazoline Containing Thieno [2, 3-*d*] Pyrimidine based derivatives 5(a-j) According to the reported procedure [**XLI**].

Characterization:

The IR spectrum of 5 a-j exhibited a band due to =CH str. (3100-3000 Cm^{-1}), C=C str. (1635-1495 Cm^{-1}), N-O str. (1350 & 1540 Cm^{-1}), 1650 (C=N, pyrazoline ring) stretching vibrations.

Further, in their 1H NMR (DMSO) spectrum, the appearance of a signal at δ 3.8-3.9 (dd, 1H, Hx pyrazoline), 3.62-3.56 (dd, 1H, HB pyrazoline) and 3.90-3.83 (dd, 1H, HA pyrazoline) confirms the presence of the pyrazoline ring.

The chemical shifts of the final compounds carbon chemical shifts are vary from δ = 195 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. The carbon chemical shift of the aldehyde carbon at δ= 195 ppm.

Readily available starting materials and simple synthesizing procedures make this method very attractive and convenient for the synthesis of pyrazoline derivatives(5a-5j). Formation of products was confirmed by recording their ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectra.

The IR spectra of new chalcones confirmed by the presence of two stretching bands at 1660-1590, this due to C=O and CH=CH, in addition the ^{1}HNMR of chalcone have two doublet signal one at 7.40 ppm which belong CHβ, and one at 6.90 which belong CHα. upon cyclocondensation of chalcones with hydrazine hydrate it produce new pyrazoline derivatives 5(a-j) respectively, where the spectral data confirm the existence of this cyclo condensation reaction where the ^{1}HNMR of new pyrazolines contain

two signal one at 3.6 & 3.9 ppm due to two hydrogens of (C-4) in pyrazoline ring and another one at 3.95 ppm due one hydrogen of (C-5) in pyrazoline ring that prove the cyclocondensation well occur. in addition the disappearance of sharp stretching of carbonyl and appearance of starching at 1640 (C=N of pyrazoline) and all this fact will prove this cyclocondensation reaction and formation of pyrazoline ring with good yield 65-85%. The structures of some the compounds were established from the spectral data of the resulting compounds.

Anti microbial activity 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 1&2.** From anti-bacterial and anti-fungal activity screening results, it has been observed that compounds 5e, 5d, 5i and 5g possess good activity.

Conclusion

The synthesized 2-pyrazolines 5a-j all are novel. We have synthesized a series of new pyrazolines 5a-j containing bioactive hetero aryl pharmacophore such as Thieno [2, 3*,-d*] Pyrimidine using convenient method. The antimicrobial activity of representative pyrazolines 5 a-j showed excellent antimicrobial activity. Compounds with electron withdrawing groups

such as $–CF_3$ and $–OCF_3$ groups are present in two moieties exhibited best antimicrobial activity. The data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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