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SYNTHESIS AND ANTI-MICROBIAL STUDIES OF NOVEL ISOXAZOLINE DERIVATIVES BEARING THIENO [2, 3-*D*] PYRIMIDINE AS A CORE UNIT

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Abstract: A new series of 5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-p-substituted-4,5-dihydroisoxazole derivatives (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 hydroxylamine hydrochloride 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.

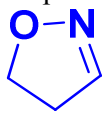
Keywords: Chalcones, Thieno-pyrimidines, 2-Isoxazolines, Antimicrobial activity.

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 and oxygen containing ring system, like pyrazoline and isoxazoline mainly due to their higher pharmacological activity.

Heterocyclic Compounds have so far been Synthesized Mainly due to the wide range of Biological Activities [**I**]. Much attention has paid to the synthesis of heterocyclic compounds bearing nitrogen and oxygen containing ring system, like isoxazoline mainly due to their higher pharmacological activity. Isoxazolines posses' medicinal activities such as anti-inflammatory [**II**], anti-bacterial, anti-convulsant [**III**], anti-biotic [**IV**], anti-tubercular [**V**], anti-fungal [**VI**] and anxiolytic activity [**VII**]. The Isoxazole nucleus is a prominent structural moiety found in numerous natural products and synthetic compounds with vital medicinal value [**VIII**], also possess as anti-Influenza virus activity [**IX**]. Isoxazoline derivatives controlled botrytis cinera on cucumbers [**X**] has been found to have antiviral properties against herpes type 2 viruses [**XI**]. Penicillin derivatives containing isoxazole ring are found

to be antibacterial [**XII]**. Isoxazole derivatives are used as corrosion inhibitors for fuels and lubricants [**XIII**].Its derivatives also show a good potency in animal models of thrombosis [**XIV].** In addition, isoxazoline derivatives have played a crucial role as intermediates in the organic synthesis of number of heterocyclic pharmacological active compounds.



Isoxazoline

Fig 1 Structure of Isoxazoline

Chalcones; either natural or synthetic are well known to exhibit promising biological activities. Due to their interesting pharmacological activities, including antioxidant [**XV,XVI**], antibacterial [**XVII**], anti-leishmanial [**XVIII**], anti-cancer [**XIX**], anti-angiogenic [**XX**], anti-infective, anti-inflammatory [**XXI**], anti-fungal [**XXII**], anti-malarial [**XXIII**], anti-tumor [**XXIV**], anti-protozoal [**XXV]** and cytotoxic properties [**XXVI**].

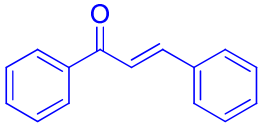
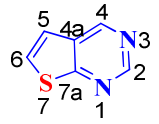


Fig.2 General structure of Chalcones

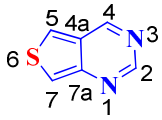
The chalcones are unsaturated ketones containing the reactive keto ethylene group

Thieno Pyrimidine is a bi cyclic heterocyclic compound consists of a five membered thiophene ring is fused to a six membered hetero cylic 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.

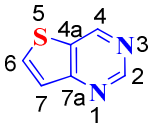
Synthetic approaches for the construction of a number of thieno Pyrimidines are well established. There exists three possible types of fusion of thiophene to Pyrimidine ring results in corresponding isomeric thienopyrimidines namely**;[Fig.3]** thieno[2,3-*d*]pyrimidines (a), thieno[3,4-*d*]pyrimidines (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 thieno pyrimidine moiety are of interest because of their interesting pharmacological and biological activities [**XXVII– XXIX**]. They bear structural analogy and iso electronic relation to purine and several substituted thieno [2, 3-*d*] Pyrimidine derivatives shown to exhibit prominent and versatile biological activities [**XXX, XXXI**]. 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 [**XXXII**], analgesic [**XXXIII**], antimicrobial [**XXXIV, XXXV**] and antiviral agents [**XXXVI**].

Some reviews on Pyrimidine thiones [**XXXVII**] and condensed pyrimidines, namely pyrazolo-pyrimidines [**XXXVIII**] and furo-pyrimidines [**XXXIX**].Thieno-pyrimidines are interesting heterocyclic compounds and a number of derivatives of these compounds display therapeutic activity as antimicrobial [**XL-XLIII**], anti-viral [**XLIV-XLV**], anti-inflammatory [**XLVI-XLVII**], anti-diabetic [**XLVIII**], anti-oxidant [**XLIX**], anti-tumour [**L-LIV**] and anti-cancer agents [**LV-LVI**], anti-depressant [**LVII**], anti-platelet [**LVIII**], anti-hypertensive [**LIX**], herbicidal [**LX**] and plant growth regulatory properties [**LXI**].

Encouraged by the diverse biological activities of isoxazoline derivatives, it was decided to prepare a new series of isoxazoline derivatives with thieno [2, 3-*d*] Pyrimidine as a core unit.

In the present communication, chalcones (4a-j) were prepared by the action of substituted acetophenones (3a-j) with thieno [2, 3-*d*] pyrimidine-6-carbaldehyde (2) 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 hydroxyl amine hydrochloride in presence of pyridine to obtained isoxazoline derivatives (5a-j) Scheme 1. The structures of all synthesized compounds were assigned on the basis of IR, Mass, 1H NMR spectral data and elemental analysis. Further these compounds were subjected for antifungal and antibacterial activity.

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 & ^{13}C NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

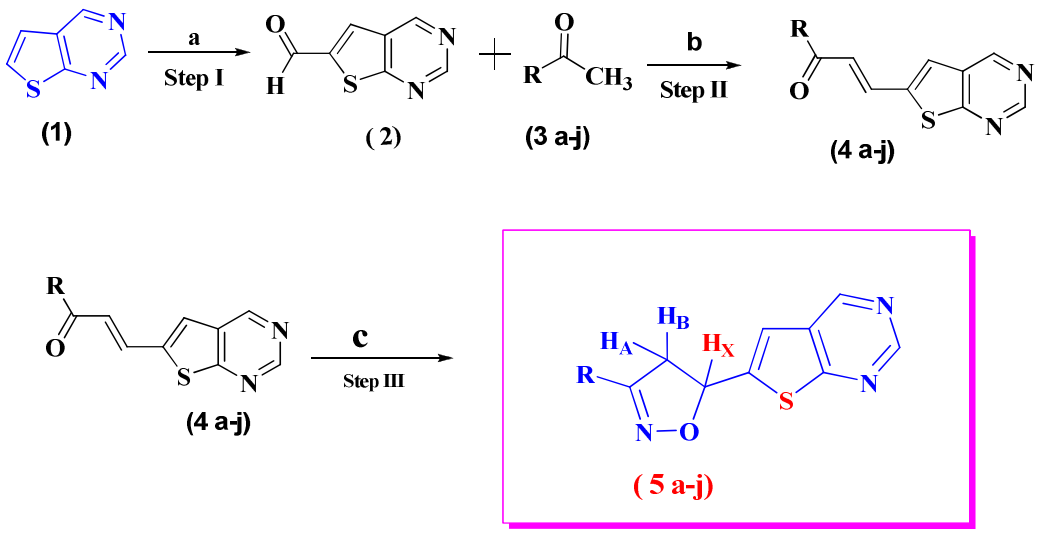
Materials and methods

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

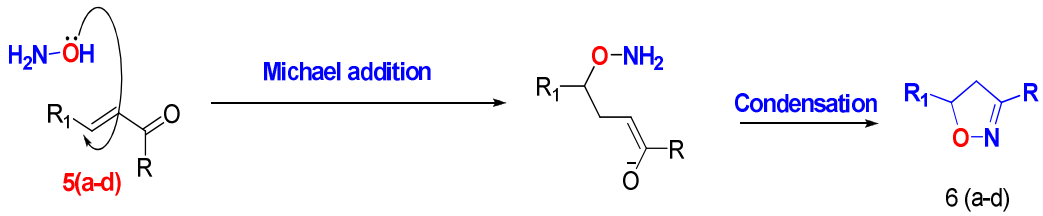


R = -Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 tri fluoro methoxy phenyl, -4 Tri fluoro phenyl, -4 Nitro phenyl, - pyrazin-2-yl, - pyridin-4-yl, Thiophene 2-yl, Furan-2-yl acetyl groups.

Reagents and Reaction conditions: (a) *DMF,$POCl_3$,80^{O}C,4hrs* (b) *NaOH,Ethanol, RT, 24 hrs* (c) *Hydroxylamine hydrochloride (***NH_2-OH.HCl***) , pyridine, Reflux, 2hrs.*

Scheme: Synthetic path way of preparation of Novel isoxazolines containing Thieno [2, 3-*d*] Pyrimidine Nucleus (5 a-j).

Possible Mechanism For Formation of Isoxazoline 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 ^{1}H 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 (^{1}H 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 (^{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).

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 $0^{O}C$, $POCl_3$ (15 mL) was added drop wise for 30 min. and stirring continued for 4 h at $80^{o}C$ 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.

^{1}H 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=), 7.95 (d, 1H, β C-H), 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=), 7.95 (d, 1H, β C-H), 7.98(2H,d), 7.4(2H,d), 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=), 7.95 (d, 1H, β C-H), 8.25(2H, d), 7.2(2H, d), 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=), 7.95 (d, 1H, β C-H), 8.25(2H, d), 7.2(2H,d).
^{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 9.4 (S, 1H, Ar-H), 8.8 (S, 1H, - Ar-H), 8.23(S,1H), 7.55 (d, 1H, CO-CH=), 7.90 (d, 1H, β C-H), 8.1(2H,d), 7.8(2H,d).
^{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 9.4 (S, 1H, Ar-H), 8.8 (S, 1H, - Ar-H), 8.23(S,1H), 7.55 (d, 1H, CO-CH=), 7.95 (d, 1H, β C-H), 8.2(2H,d), 8.5(2H,d).
^{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$(M-H)^+$.

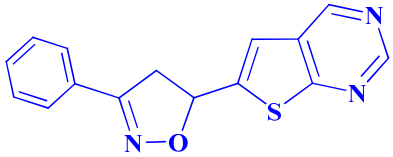
(E)-1-(pyridin-4-yl)-3-(thieno [2, 3-*d*] pyrimidin-6-yl) prop-2-en-1-one (4h):
Yield: 82% (yellow colour solid);
IR (KBr, cm^{-1}): 3100(Ar CH), 1670 (C=O Stretching), 1655(C=C Stretching), 1460(C=N), 685(C-S-C).
^{1}H NMR (400 MHz; $CDCl_3$): δH 9.34(S, 1H, Ar-H), 8.82 (S, 1H, - Ar-H), 8.25(S, 1H), 6.75 (d, 1H, CO-CH=), 7.55 (d, 1H, β C-H), 8.90(1H, d), 8.12(1H, d).
^{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=), 7.68 (d, 1H, β C-H), 8.12(1H, d), 8.26(1H, d), 7.42(1H, t).
^{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=), 7.65 (d, 1H, β C-H), 8.12(1H, d), 7.92(1H, t), 8.72(1H, d).
^{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$(M-H)^+$.

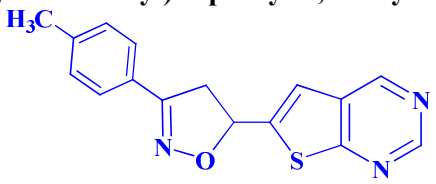
General procedure for the synthesis of 2-Isoxazoline (5a-j):
A mixture of chalcones (**0.01 moles**) and hydroxylamine hydrochloride (**0.1 moles**) in 25 mL pyridine was refluxed for 2hrs. On cooling the reaction mixture was poured over crushed ice and conc. HCl. The solid obtained was filtered, washed with water and crystallized from ethanol.

3-phenyl-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4,5-dihydroisoxazole (5a):



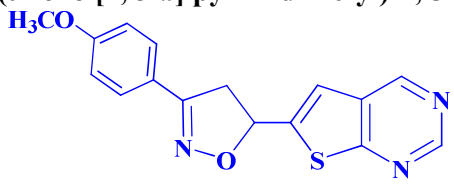
structure of 3-phenyl-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazole (5a)

Yield: 75% (white colour solid);
m.p.146^{O}C.
IR (KBr, cm^{-1}): 3140(-Ar CH), 1612 (C=N of isoxazoline ring), 1540 (C=C), -C-O-N (1231 of isoxazoline).
^{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=11.4 Hz, HB), 4.92 (dd, 1H, J=12.2, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 43, 80,125,130,143,147.73, 150, 155.5.
MS (70 ev): m/z = 304(M+Na)$^{+}$.
5-(thieno [2, 3-*d*] pyrimidin-6-yl)-3-p-tolyl-4,5-dihydroisoxazole (5b):

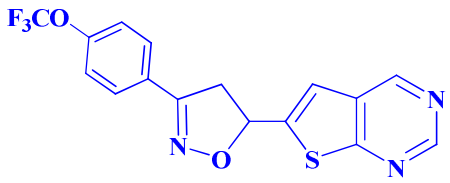


structure of 5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-*p*-tolyl-4,5-dihydroisoxazole (5b)

Yield: 80% (white colour crystals);
m.p.134^{O}C.
IR (KBr, cm^{-1}): 3110(-Ar CH), 2990(SP^3 CH), 1615 (C=N of isoxazoline ring), 1560 (C=C), -C-O-N (1241 of isoxazoline).
^{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), 3.85 (dd, 1H, J=10.6 Hz, HA), 3.65 (dd, 1H, J=11.4 Hz, HB), 4.65 (dd, 1H, J=12.2, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 23, 43, 80,125,130,143,147.73, 150, 158.5.
MS (70 ev): m/z = 296(M+H)$^{+}$.
3-(4-methoxyphenyl)-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4, 5-dihydroisoxazole (5c):



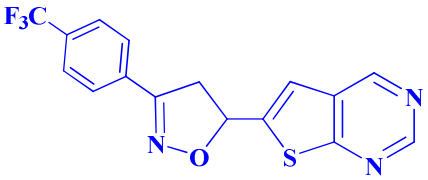
structure of 3-(4-methoxyphenyl)-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazole (5c)
Yield: 90% (off white colour solid);
m.p.146^{O}C.
IR (KBr, cm^{-1}): 3120(-Ar CH), 1615 (C=N of isoxazoline ring), 1165(-OCH$_3$), 1540 (C=C), -C-O-N (1231 of isoxazoline), 668(C-S-C).
^{1}H NMR (400 MHz; DMSO-d$_6$): 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), 4.69 (dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 43, 58, 80, 115,120,125, 128.92, 124.03,135, 149,151.67, 154.75, 159.62, 163.1.
MS (70 ev): m/z = 312(M+H)$^{+}$.
5-(thieno [2, 3-*d*] pyrimidin-6-yl)-3-(4-(trifluoromethoxy) phenyl)-4, 5-dihydroisoxazole (5d):



structure of 5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-(4-(trifluoromethoxy)phenyl)-4,5-dihydroisoxazole (5d)

Yield: 90% (light yellow colour solid);
m.p.186^{O}C.
IR (KBr, cm^{-1}): 3110(-Ar CH), 1610 (C=N of isoxazoline ring), 1155 (-OCH_3), 1545 (C=C), -C-O-N (1240 of isoxazoline), 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), 4.69 (dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 82, 120,125, 128.92, 124.03,135, 149,151.67, 154.75, 156.62, 159.23.
MS (70 ev): m/z = 366.015 $(M+H)^{+}$.

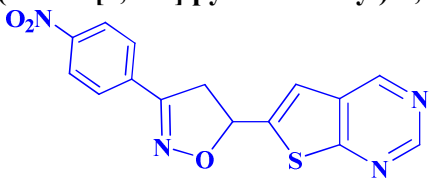
5-(thieno [2, 3-*d*] pyrimidin-6-yl)-3-(4-(trifluoromethyl) phenyl)-4, 5-dihydroisoxazole (5e):



structure of 5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-(4-(trifluoromethyl)phenyl)-4,5-dihydroisoxazole (5e)

Yield: 89% (yellow colour solid);
m.p.125^{O}C.
IR (KBr, cm^{-1}): 3130(Ar CH), 1612 (C=N of isoxazoline ring), 1540 (C=C), -C-O-N (1231 of isoxazoline), 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), 4.69 (dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 46, 85, 120,125, 128.72, 124.13,135, 149,151.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 350.04$(M+H)^{+}$.

3-(4-nitrophenyl)-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4, 5-dihydroisoxazole (5f):

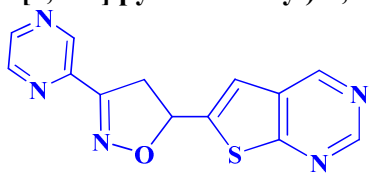


structure of 3-(4-nitrophenyl)-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazole (5f)

Yield: 80% (yellow colour solid);
m.p.166^{O}C.
IR (KBr, cm^{-1}): 3110(Ar CH), 1625 (C=N of isoxazoline ring), 1545 (C=C), -C-O-N (1236 of isoxazoline), 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), 4.70(dd, 1H, J=12.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 46, 85, 125, 128.72, 135, 143,145, 150.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 325.047(M-H)$^{+}$.

3-(pyrazin-2-yl)-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4, 5-dihydroisoxazole (5g):

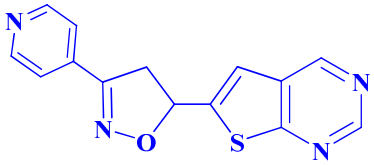


structure of 3-(pyrazin-2-yl)-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazole (5g)

Yield: 75% (off white colour solid);
m.p.136^{O}C.
IR (KBr, cm^{-1}): 3100(Ar CH), 1625 (C=N of isoxazoline ring), 1540 (C=C), -C-O-N (1230 of isoxazoline), 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), 4.60(dd, 1H, J=13.2Hz, Hx).

^{13}C NMR (100 MHz; DMSO-d_6): δC 46, 85, 125, 128.72, 135, 143,145, 150.67, 154.75, 156.62, 158.23.
MS (70 ev): m/z = 282.05(M-H)$^{+}$.

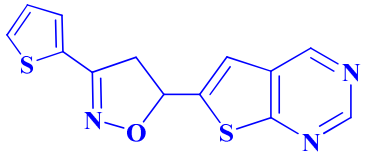
3-(pyridin-4-yl)-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4, 5-di hydro isoxazole (5h):



structure of 3-(pyridin-4-yl)-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazolele (5h)

Yield: 74% (yellow colour solid);
m.p.178^{O}C.
IR (KBr, cm^{-1}): 3120(Ar CH), 1645 (C=N of isoxazoline ring), 1560 (C=C), 1236(-C-O-N of isoxazoline), 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), 4.60(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d_6): δC 45, 84, 125, 128.72, 138, 144,148, 156.62, 159.23.
MS (70 ev): m/z = 281(M-H)$^{+}$.

5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (5i) :

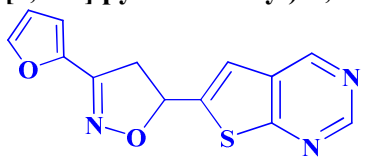


structure of 5-(thieno[2,3-*d*]pyrimidin-6-yl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (5i)

Yield: 80% (pale yellow colour solid);
m.p.168^{O}C.

IR (KBr, cm^{-1}): 3100(Ar CH), 1635 (C=N of isoxazoline ring), 1560 (C=C), 1236(-C-O-N of isoxazoline), 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), 4.60(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 45, 81, 125, 128.72,143, 147,149,157.
MS (70 ev): m/z = 286.36(M-H)$^{+}$.

3-(furan-2-yl)-5-(thieno [2, 3-*d*] pyrimidin-6-yl)-4, 5-dihydroisoxazole (5j) :



structure of 3-(furan-2-yl)-5-(thieno[2,3-*d*]pyrimidin-6-yl)-4,5-dihydroisoxazole (5j)

Yield: 84% (off white colour solid);
m.p. 155^{O}C.
IR (KBr, cm^{-1}): 3110(Ar CH), 1655 (C=N of isoxazoline ring), 1565 (C=C), 1230(-C-O-N of isoxazoline), 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), 4.60(dd, 1H, J=13.2Hz, Hx).
^{13}C NMR (100 MHz; DMSO-d$_6$): δC 45, 81, 125, 128.72,143, 147,149,157.
MS (70 ev): m/z = 270.042(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 [**LXII, LXIII**]. 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>5h>5f>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		**Staphylococcus 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	9.5	7	9.5	7.5	12	10	12.5	10.5
5g	11	9.5	11.5	8.5	12.5	12	13	11.5
5h	10	8	11.1	9.5	12	11	13.5	11
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**)	---	---	----	-----	----	-----	-----	-----

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against **Candida albicans** and **Aspergillus flavus** in DMSO by agar diffusion method [**LXIV**]. 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	9.5	7	9.5	7.5
5g	11	9.5	11.5	8.5
5h	10	8	11.1	9.5

5i	13	11.5	10.5	8
5j	8.5	6.5	9.0	6.5
Flucanazole	21	16	18.5	14
Control (**DMSO**)	---	---	---	---

Results and Discussion:

Chemistry:

The Title Compounds Novel isoxazolines 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 [**LXV**]. 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 [**LXVI**], these are further reacted with hydroxyl amine hydro chloride to get target novel isoxazoline Containing Thieno [2, 3-*d*] Pyrimidine based derivatives 5(a-j) According to the reported procedure [**LXVII**].

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}), C=N (ring) (1650-1580 Cm^{-1}) stretching vibration band which indicates the presence of the isoxazoline ring.

Further, in their 1H NMR (DMSO) spectrum, the appearance of a signal at δ 5.25-5.18 (dd, 1H, Hx isoxazoline), 3.62-3.56 (dd, 1H, HB isoxazoline) and 2.90-2.83 (dd, 1H, HA isoxazoline) confirms the presence of the isoxazoline ring. 3.62-3.56 (dd, 1H, HB isoxazoline) and 2.90-2.83 (dd, 1H, HA isoxazoline) confirms the presence of the isoxazoline 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 isoxazoline derivatives. 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 hydroxylamine it produce new isoxazoline derivatives 5(a-j) respectively, where the spectral data confirm the existence of this cyclocondensation reaction where the ^{1}HNMR of new isoxazolines contain two signal one at 3.6 & 3.8 ppm due to two hydrogens of (C-4) in isoxazoline ring and another one at 4.65 ppm due one hydrogen of (C-5) in isoxazoline ring that prove the cyclocondensation well occur. in addition the disappearance of sharp stretching of carbonyl and appearance of starching at 1590 (C=N of isoxazoline) and all this fact will prove this cyclocondensation reaction and formation of isoxazoline 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**. 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-isoxazoline 5a-j all are novel. We have synthesized a series of new isoxazolines 5a-j containing bioactive hetero aryl pharmacophore such as Thieno [2, 3*,-d*] Pyrimidine using convenient method. The antimicrobial activity of representative isoxazolines 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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