Antibacterial Screening of some Heterocyclic Compounds Containing Quinoline Nucleus

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Abstract. In this work, some novel derivatives of Cyanopyridines and Isoxazoles were synthesized using Vilsmeier-Haack reagent and their structures were confirmed by FTIR, ^{1}H NMR and mass spectroscopic methods. The antibacterial activities of these synthesized compounds were studied in DMSO and DMF by agar well diffusion method against some Gram positive and Gram negative bacteria. It is observed that activity depends upon three S: solvent, strain and structure.

Introduction

Quinolines are heterocyclic nitrogen containing basic compounds in which the pyridine ring is electron deficient. Therefore, nucleophilic attack takes place at the 2- and 4-positions. Quinolines and their derivatives are very important compounds because of their wide occurrence in natural products [1, 2] and biologically active compounds [3-8]. These derivatives are also used for the preparation of nano- and meso structures having enhanced electronic and photonic properties [9, 10] and are known to have applications as pharmaceuticals, agrochemicals and synthetic building blocks [11]. Quinolines have been found to possess antimalarial, anti-bacterial, antifungal, antihelmintic, cardiotonic, anticonvulsant, anti-inflammatory, and analgesic activity [12-18].

Recently, more and more new simple and elegant syntheses of substituted quinolines have been described [19-25]. The synthesis of quinoline derivatives thus continues to be an attractive area of research and the synthesis of various substituted quinolines have been largely described in the literature through different strategies [26-28]. Some of these substituted quinolines are important constituents of several pharmacologically active compounds. A number of biological activities [29-34] have also been associated with quinoline containing compounds such as anti-inflammatory, antiallergic, antimalarial, antibacterial, antiproliferative, anticancer and antiparasitic activities.

Thus, aim of the present work is to synthesize some new nitrogen containing heterocyclic compounds and to study their antibacterial activity. Some novel derivatives of cyanopyridine and isoxazole bearing quinoline nucleus are synthesized and their structures were elucidated on the basis of IR, MASS and 1H NMR spectral data. All the compounds have been screened for their in-vitro biological activity by agar well diffusion method.

Materials and Methods

The chemicals used in the present work were 4-fluoroaniline, acetic anhydride, sodium bicarbonate, acetophenone, p-methoxy-acetophenone, p-nitro acetophenone, p-amino-acetophenone, p-bromo-acetophenone, m-nitro-acetophenone, p-hydroxy-acetophenone, p-chloro-acetophenone, o-hydroxy-acetophenone, malononitrile, ethanol, POCl_3, sodium hydroxide, ammonium acetate, sodium acetate, acetic acid, DMF, DMSO, hydroxylamine hydrochloride etc. All these chemicals were purchased from Spectrochem (Mumbai, India) and were of LR grade. These chemicals were used as such for synthesis.

Test Microorganisms: The microorganisms used in the present study were four Gram positive and four Gram negative bacteria.

Gram positive bacteria: *Bacillus cereus* ATCC11778, *Micrococcus flavus* ATCC10240, *Staphylococcus epidermidis* ATCC12228, and *Staphylococcus aureus* ATCC29737.

Gram negative bacteria: *Proteus mirabilis* NCIM2241, *Salmonella typhimurium* ATCC23564, *Citrobacter freundii* ATCC10787 and *Klebsiella pneumoniae* NCIM2719.

Microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India and were maintained at 4°C on nutrient agar slants.
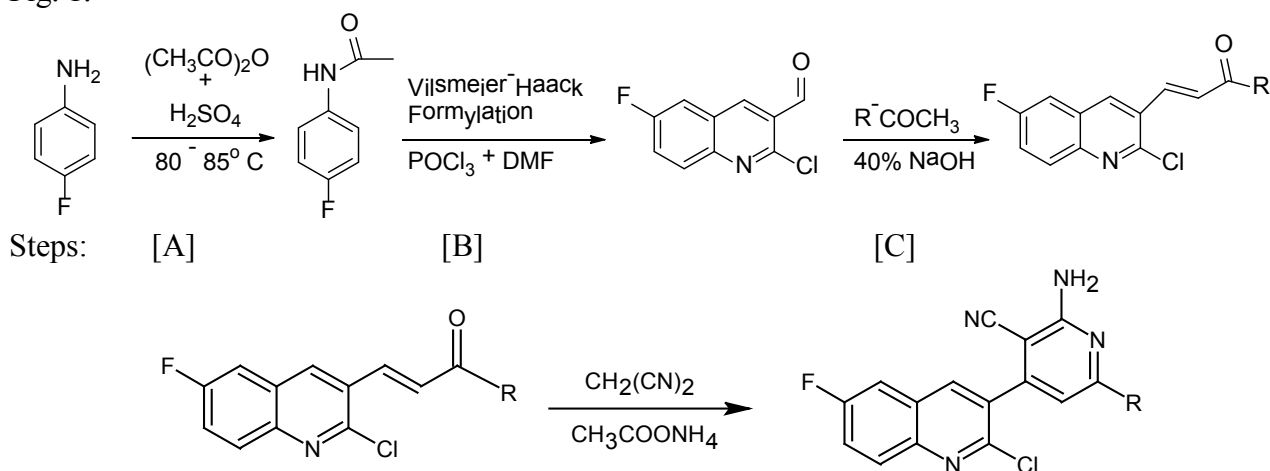
Synthesis:

Cyanopyridines:

Synthesis of 2-amino-4-(2-chloro-6-fluoroquinolin-3-yl)-6-(4-methoxy phenyl) nicotine nitrile.

Synthesis of *N*-(4-fluorophenyl)acetamide: Equimolar mixture of 4-fluoroaniline and acetic anhydride (0.01M) in ethanol was refluxed in oil bath for 2-3 hrs using H_2SO_4 as catalyst. The crude product was isolated and crystallized from absolute ethanol.

Synthesis of 2-chloro-6-fluoroquinoline-3-carbaldehyde: *N*- (4-fluorophenyl) acetamide prepared above was added in a mixture of Vilsmeier-Haack reagent (prepared by drop wise addition of 6.5 ml $POCl_3$ in ice cooled 2 ml DMF) and was refluxed for 27 hrs on oil bath. After the completion of reaction, reaction mixture was poured into ice followed by neutralization using saturated aqueous sodium bicarbonate solution. The crude product was isolated by filtration and was crystallized from ethanol.

Synthesis of (2*E*)-3-(2-chloro-6-fluoroquinolin-3-yl)-1-(4-methoxy phenyl) prop-2-en-1- one: An equimolar solution of 2-chloro-6-fluoroquinoline-3-carbaldehyde and p-methoxy-acetophenone was prepared in ethanol. The solution was made alkaline by 40% NaOH aqueous solution. The reaction mixture was stirred for 48 hrs. The contents were poured into ice and acidified with 50% HCl solution. The obtained crude product was filtered and was crystallized from ethanol.

Synthesis of 2-amino-4-(2-chloro-6-fluoroquinolin-3-yl)-6-(4-methoxyphenyl) nicotine nitrile (NCP-1): To an equimolar mixture of (2*E*) - 3- (2- chloro- 6-fluoro quinolin-3-yl) -1-(4- methoxy phenyl) prop-2-en-1-one and malononitrile in ethanol, ammonium acetate was added and solution was refluxed for 10-12 hrs in oil bath. The content was poured on crushed ice. The obtained product was filtered, washed with water and crystallized from ethanol. The reaction scheme is given in Fig. 1.
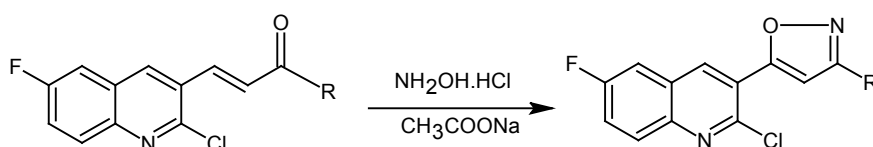

Figure 1. Reaction scheme for the synthesis of cyanopyridines.

Similarly, ten other substituted cyanopyridines have been prepared.

Isoxazoles:

Synthesis of (2*E*)-3-(2-chloro-6-fluoroquinolin-3-yl)-1-(4-methoxyphenyl) prop-2-en-1- one: It is given above.

Synthesis of 2-chloro-6-fluoro-3-[3-(4-methoxyphenyl) isoxazol-5-yl]quinoline (NISO-1): To an equimolar solution of anhydrous sodium acetate and hydroxylamine hydrochloride in ethanol, minimum amount of hot acetic acid and solution of (2*E*)-3-(2-chloro-6-fluoro quinolin -3-yl)-1-(4-methoxy phenyl) prop-2-en-1-one in ethanol was added. The mixture was refluxed on a water bath for 10 hrs. The product was isolated and recrystallized from ethanol. The reaction is in Fig. 2.

Figure 2. Reaction scheme for the synthesis of isoxazoles.

Similarly, other substituted isoxazoles have been prepared. All the synthesized compounds were re crystallized prior to use.

Spectral analysis: The structure of all the synthesized compounds was confirmed by spectroscopic techniques.
The IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. The Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR was determined in DMSO solution on a Bruker Ac 400 MHz spectrometer.

Antibacterial studies: The antibacterial activities of all synthesized compounds were studied in DMF and DMSO. Both the solvents were fractionally distilled by standard method [35].
In the present work, the antibacterial activities of the synthesized compounds have been screened against some Gram positive and Gram negative bacterial stains using agar well diffusion method.

Preparation of test compounds: The solutions were prepared at a concentration of 1 mg/μl for all the compounds.

Preparation of the plates and microbiological assay: The antibacterial evaluation was done by agar well diffusion method [36, 37] using Mueller Hinton Agar No.2 as the nutrient medium. The agar well diffusion method was preferred to be used in this study because it was found to be better than the disc diffusion method as suggested by Parekh et al. [37]. The bacterial strains were activated by inoculating a loop full of test strain in 25 ml of N-broth and the same was incubated for 24 h in an incubator at 37° C. 0.2 ml of the activated strain was inoculated in Mueller Hinton Agar. Mueller Hinton Agar kept at 45°C was then poured in the Petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 24 h at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain and each solvent. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of these synthesized compounds.

Results

In both Cyanopyridine and Isoxazole series, ten compounds are synthesized. The synthesized compounds were purified through column using chloroform: methanol (80:20 v/v). The purity of compounds was checked by TLC using appropriate solvent systems.
The physical parameters of synthesized cyanopyridine and isoxazole compounds are listed in Tables 1 and 2.

Table 1. Physical constants of cyanopiridines.

Sr. No.	Compound Code	R	R_f* Value	M.P. °C	Yield %
1	NCP-1	4-OCH$_3$-C$_6$H$_4$-	0.65	212	69
2	NCP-2	4-NH$_2$-C$_6$H$_4$-	0.51	210	72
3	NCP-3	4-Br-C$_6$H$_4$-	0.49	225	75
4	NCP-4	4-NO$_2$-C$_6$H$_4$-	0.75	196	71
5	NCP-5	3-NO$_2$-C$_6$H$_4$-	0.69	178	63
6	NCP-6	4-OH-C$_6$H$_4$-	0.73	188	67
7	NCP-7	4-CH$_3$-C$_6$H$_4$-	0.52	162	64
8	NCP-8	4-Cl-C$_6$H$_4$-	0.58	154	75
9	NCP-9	2-OH-C$_6$H$_4$-	0.47	202	79
10	NCP-10	C$_6$H$_5$-	0.45	187	81

*Ethyl acetate:Hexane: 2.5:7.5

Table 2. Physical constants of isoxazoles.

Sr. No.	Compound Code	R	R_f* Value	M.P. °C	Yield %
1	NISO-1	4-OCH$_3$-C$_6$H$_4$-	0.57	171	71
2	NISO-2	4-NH$_2$-C$_6$H$_4$-	0.68	184	65
3	NISO-3	4-Br-C$_6$H$_4$-	0.65	210	69
4	NISO-4	4-NO$_2$-C$_6$H$_4$-	0.61	168	72
5	NISO-5	3-NO$_2$-C$_6$H$_4$-	0.72	241	75
6	NISO-6	4-OH-C$_6$H$_4$-	0.75	222	64
7	NISO-7	4-CH$_3$-C$_6$H$_4$-	0.53	198	61
8	NISO-8	4-Cl-C$_6$H$_4$-	0.68	212	64
9	NISO-9	2-OH-C$_6$H$_4$-	0.71	202	67
10	NISO-10	C$_6$H$_5$-	0.78	235	58

*Ethyl acetate: Hexane: 2:8

Spectral data

Cyanopyridines:

NCP-1 IR (cm^{-1}, KBr): 2933.83 (asym. C-H str.), 2841.24 (sym. C-H str.), 1456.30 (asym. C-H def.), 1363.72 (sym. C-H def.), 3061.13 (sym. aromatic C-H str.), 1508.38 (aromatic C=C str.), 1658.84 (C=N str.), 1247.99 (C-N str.), 2208.57 (nitrile C-N str.), 3323.46 (N-H str.), 1600.97 (N-H def.), 1220.98 (asym. C-O-C str.), 1026.16 (sym. C-O-C str.), 1174.69 (C-F str.), 661.61 (C-Cl str.), **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 3.92 (3H, singlet, -OCH$_3$), 7.09-7.12 (2H, doublet, Ar-CH), 7.50-7.54 (1H, triplet, Ar-CH), 7.66-7.69 (1H, doublet, Ar-CH), 7.75-7.77 (1H, doublet, Ar-CH), 7.84-8.11 (4H, multiplet, Ar-CH), **MS: (m/z)** = 404.8

NCP-2 IR (cm^{-1}, KBr): 2933.22 (asym. C-H str.), 2841.28 (sym. C-H str.), 1456.10 (asym. C-H def.), 1363.60 (sym. C-H def.), 3061.17 (sym. aromatic C-H str.), 1521.04 (aromatic C=C str.), 1658.42 (C=N str.), 1247.41(C-N str.), 2232.14 (nitrile C-N str.), 3368.81 (N-H str.), 1600.93 (N-H def.), 1220.91 (asym. C-O-C str.), 1026.10(sym. C-O-C str.), 1174.61 (C-F str.), 721.40 (C-Cl str.), **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.04-7.10 (2H, doublet, Ar-CH), 7.51-7.53 (1H, triplet, Ar-CH), 7.65-7.67 (1H, doublet, Ar-CH), 7.74-7.76 (1H, doublet, Ar-CH), 7.85-8.10 (4H, multiplet, Ar-CH), 7.93 (2H, singlet, -NH$_2$), **MS: (m/z)** = 389.8

NCP-3 IR (cm^{-1}, KBr): 2933.81 (asym. C-H str.), 2841.35 (sym. C-H str.), 1456.12 (asym. C-H def.), 1363.42 (sym. C-H def.), 3061.14 (sym. aromatic C-H str.), 1508.35 (aromatic C=C str.), 1658.81 (C=N str.), 1247.87 (C-N str.), 2208.51 (nitrile C-N str.), 3344.22 (N-H str.), 1600.55 (N-H def.), 1220.32 (asym. C-O-C str.), 1026.19 (sym. C-O-C str.), 1174.64 (C-F str.), 712.49 (C-Cl str.),

^{1}H NMR (DMSO-d${}_6$) *δ(ppm)***:** 7.06-7.10 (2H, doublet, Ar-CH), 7.52-7.53 (1H, triplet, Ar-CH), 7.62-7.65 (1H, doublet, Ar-CH), 7.73-7.76 (1H, doublet, Ar-CH), 7.82-8.05 (4H, multiplet, Ar-CH), *MS: (m/z)* = 453.7

NCP-4 *IR (cm^{-1}, KBr)***:** 2933.66 (asym. C-H str.), 2841.34 (sym. C-H str.), 1456.27 (asym. C-H def.), 1363.98 (sym. C-H def.), 3061.47 (sym. aromatic C-H str.), 1508.75 (aromatic C=C str.), 1658.74 (C=N str.), 1247.94 (C-N str.), 2221.74 (nitrile C-N str.), 3323.27 (N-H str.), 1600.77 (N-H def.), 1220.64 (asym. C-O-C str.), 1026.04 (sym. C-O-C str.), 1174.41 (C-F str.), 724.33 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 7.04-7.09 (2H, doublet, Ar-CH), 7.52-7.58 (1H, triplet, Ar-CH), 7.63-7.68, (1H, doublet, Ar-CH), 7.72-7.75 (1H, doublet, Ar-CH), 7.80-8.09 (4H, multiplet, Ar-CH), *MS: (m/z)* = 419.8

NCP-5 *IR (cm^{-1}, KBr)***:** 2933.72 (asym. C-H str.), 2841.37 (sym. C-H str.), 1456.74 (asym. C-H def.), 1363.87 (sym. C-H def.), 3061.41(sym. aromatic C-H str.), 1508.55 (aromatic C=C str.), 1658.44 (C=N str.), 1247.77 (C-N str.), 2234.37 (nitrile C-N str.), 3315.57 (N-H str.), 1600.97 (N-H def.), 1220.98 (asym. C-O-C str.), 1026.54 (sym. C-O-C str.), 1174.27 (C-F str.), 668.32 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 7.07-7.10 (2H, doublet, Ar-CH), 7.50-7.54 (1H, triplet, Ar-CH), 7.66-7.69 (1H, doublet, Ar-CH), 7.75-7.77 (1H, doublet, Ar-CH), 7.84-8.11 (3H, multiplet, Ar-CH), 8.26 (1H, singlet, Ar-CH), *MS: (m/z)* = 419.8

NCP-6 *IR (cm^{-1}, KBr)***:** 2933.77 (asym. C-H str.), 2841.47 (sym. C-H str.), 1456.64 (asym. C-H def.), 1363.34 (sym. C-H def.), 3061.24 (sym. aromatic C-H str.), 1508.79 (aromatic C=C str.), 1658.65 (C=N str.), 1247.93 (C-N str.), 2232.24 (nitrile C-N str.), 3298.77 (N-H str.), 1600.54 (N-H def.), 1220.47 (asym. C-O-C str.), 1026.33 (sym. C-O-C str.), 1174.47 (C-F str.), 668.34 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 7.02-7.10 (2H, doublet, Ar-CH), 7.55-7.57 (1H, triplet, Ar-CH), 7.67-7.69 (1H, doublet, Ar-CH), 7.76-7.78 (1H, doublet, Ar-CH), 7.86-8.13 (4H, multiplet, Ar-CH), 9.12 (1H, singlet, -OH), *MS: (m/z)* = 390.8

NCP-7 *IR (cm^{-1}, KBr)***:** 2933.43 (asym. C-H str.), 2841.57 (sym. C-H str.), 1456.77 (asym. C-H def.), 1363.78 (sym. C-H def.), 3061.17 (sym. aromatic C-H str.), 1508.64 (aromatic C=C str.), 1658.72 (C=N str.), 1247.87 (C-N str.), 2208.38 (nitrile C-N str.), 3275.87 (N-H str.), 1600.54 (N-H def.), 1220.91 (asym. C-O-C str.), 1026.24 (sym. C-O-C str.), 1174.21 (C-F str.), 678.41 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 1.25 (3H, singlet, -CH${}_3$), 7.09-7.12 (2H, doublet, Ar-CH), 7.50-7.54 (1H, triplet, Ar-CH), 7.66-7.69 (1H, doublet, Ar-CH), 7.75-7.77 (1H, doublet, Ar-CH), 7.84-8.11 (4H, multiplet, Ar-CH), *MS: (m/z)* = 388.8

NCP-8 *IR (cm^{-1}, KBr)***:** 2933.66 (asym. C-H str.), 2841.34 (sym. C-H str.), 1456.55 (asym. C-H def.), 1363.67 (sym. C-H def.), 3061.23 (sym. aromatic C-H str.), 1508.47 (aromatic C=C str.), 1658.76 (C=N str.), 1247.94 (C-N str.), 2208.24 (nitrile C-N str.), 3304.42 (N-H str.), 1600.46 (N-H def.), 1220.98 (asym. C-O-C str.), 1026.37 (sym. C-O-C str.), 1174.77 (C-F str.), 731.64 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 7.04-7.10 (2H, doublet, Ar-CH), 7.51-7.55 (1H, triplet, Ar-CH), 7.62-7.65 (1H, doublet, Ar-CH), 7.73-7.76 (1H, doublet, Ar-CH), 7.81-8.05 (4H, multiplet, Ar-CH), *MS: (m/z)* = 409.2

NCP-9 *IR (cm^{-1}, KBr)***:** 2933.47 (asym. C-H str.), 2841.75 (sym. C-H str.), 1456.11 (asym. C-H def.), 1363.52 (sym. C-H def.), 3061.44 (sym. aromatic C-H str.), 1508.78 (aromatic C=C str.), 1658.74 (C=N str.), 1247.49 (C-N str.), 2204.32 (nitrile C-N str.), 3323.78 (N-H str.), 1600.87 (N-H def.), 1220.85 (asym. C-O-C str.), 1026.17 (sym. C-O-C str.), 1174.60 (C-F str.), 711.85 (C-Cl str.), *^{1}H NMR (DMSO-d${}_6$)* *δ(ppm)***:** 7.25-7.29 (2H, doublet, Ar-CH), 7.50-7.54 (1H, triplet, Ar-CH), 7.66-7.69 (1H, doublet, Ar-CH), 7.75-7.77 (1H, doublet, Ar-CH), 7.84-8.11 (4H, multiplet, Ar-CH), 9.18 (1H, singlet, -OH), *MS: (m/z)* = 409.2

NCP-10 *IR (cm^{-1}, KBr)***:** 2933.41 (asym. C-H str.), 2841.24 (sym. C-H str.), 1456.75 (asym. C-H def.), 1363.46 (sym. C-H def.), 3061.61 (sym. aromatic C-H str.), 1508.24 (aromatic C=C str.), 1658.42 (C=N str.), 1247.87 (C-N str.), 2234.58 (nitrile C-N str.), 3285.38 (N-H str.), 1600.84 (N-H def.), 1220.79 (asym. C-O-C str.), 1026.54 (sym. C-O-C str.), 1174.85 (C-F str.), 715.58 (C-Cl str.),

^{1}H NMR (DMSO-d_6) δ(ppm): 7.33-7.36 (2H, doublet, Ar-CH), 7.50-7.54 (1H, triplet, Ar-CH), 7.66-7.69 (1H, doublet, Ar-CH), 7.75-7.77 (1H, doublet, Ar-CH), 7.84-8.11 (4H, multiplet, Ar-CH), *MS: (m/z)* = 374.8

Isoxazoles

NISO-1 *IR (cm^{-1}, KBr)*: 2899.11 (asym. C-H str.), 2845.10 (sym. C-H str.), 1456.30 (asym. C-H def.), 1423.51 (sym. C-H def.), 3020.63 (sym. aromatic C-H str.), 1506.46 (aromatic C=C str.), 1664.62 (C=N str.), 1300.07 (C-N str.), 833.28 (N-O str.), 1247.99 (asym. C-O-C str.), 1026.16 (sym. C-O-C str.), 1182.40 (C-F str.), 725.26 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 3.79 (3H, singlet, -OCH$_3$), 6.86-6.88 (2H, doublet, Ar-CH), 7.13 (1H, singlet, Ar-CH), 7.33-7.35 (2H, doublet, Ar-CH), 7.44-7.48 (1H, triplet, Ar-CH), 7.69-7.71 (1H, doublet, Ar-CH), 7.85-7.87 (1H, doublet, Ar-CH), 8.42 (1H, singlet, Ar-CH), *MS: (m/z)* = 354.8

NISO-2 *IR (cm^{-1}, KBr)*: 2899.25 (asym. C-H str.), 2845.65 (sym. C-H str.), 1456.84 (asym. C-H def.), 1423.34 (sym. C-H def.), 3020.24 (sym. aromatic 0C-H str.), 1488.52 (aromatic C=C str.), 1664.42 (C=N str.), 1311.89 (C-N str.), 824.49 (N-O str.), 1247.80 (asym. C-O-C str.), 1026.27 (sym. C-O-C str.), 1182.34 (C-F str.), 741.20 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 6.82-6.86 (2H, doublet, Ar-CH), 7.12 (1H, singlet, Ar-CH), 7.31-7.34 (2H, doublet, Ar-CH), 7.45-7.49 (1H, triplet, Ar-CH), 7.68-7.70 (1H, doublet, Ar-CH), 7.80-7.83 (1H, doublet, Ar-CH), 8.40 (1H, singlet, Ar-CH), 7.90 (2H, singlet, -NH$_2$), *MS: (m/z)* = 339.8

NISO-3 *IR (cm^{-1}, KBr)*: 2899.54 (asym. C-H str.), 2845.63 (sym. C-H str.), 1456.26 (asym. C-H def.), 1423.84 (sym. C-H def.), 3020.55 (sym. aromatic C-H str.), 1524.38 (aromatic C=C str.), 1664.38 (C=N str.), 1278.21 (C-N str.), 828.71 (N-O str.), 1247.05 (asym. C-O-C str.), 1026.04 (sym. C-O-C str.), 1182.36 (C-F str.), 725.84 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 6.81-6.83 (2H, doublet, Ar-CH), 7.10 (1H, singlet, Ar-CH), 7.32-7.36 (2H, doublet, Ar-CH), 7.46-7.49 (1H, triplet, Ar-CH), 7.70-7.73 (1H, doublet, Ar-CH), 7.83-7.85 (1H, doublet, Ar-CH), 8.40 (1H, singlet, Ar-CH), *MS: (m/z)* = 403.6

NISO-4 *IR (cm^{-1}, KBr)*: 2899.29 (asym. C-H str.), 2845.25 (sym. C-H str.), 1456.90 (asym. C-H def.), 1423.57 (sym. C-H def.), 3020.55 (sym. aromatic C-H str.), 1498.63 (aromatic C=C str.), 1664.24 (C=N str.), 1247.29 (C-N str.), 833.28 (N-O str.), 1247.57 (asym. C-O-C str.), 1026.27 (sym. C-O-C str.), 1182.34 (C-F str.), 712.79 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 6.82-6.84 (2H, doublet, Ar-CH), 7.14 (1H, singlet, Ar-CH), 7.31-7.33 (2H, doublet, Ar-CH), 7.41-7.44 (1H, triplet, Ar-CH), 7.60-7.65 (1H, doublet, Ar-CH), 7.80-7.83 (1H, doublet, Ar-CH), 8.39 (1H, singlet, Ar-CH), *MS: (m/z)* = 369.7

NISO-5 *IR (cm^{-1}, KBr)*: 2899.14 (asym. C-H str.), 2845.37 (sym. C-H str.), 1456.88 (asym. C-H def.), 1423.71 (sym. C-H def.), 3020.44 (sym. aromatic C-H str.), 1508.64 (aromatic C=C str.), 1664.62 (C=N str.), 1325.54 (C-N str.), 812.62 (N-O str.), 1247.79 (asym. C-O-C str.), 1026.10 (sym. C-O-C str.), 1182.46 (C-F str.), 724.41 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 6.89 (1H, siglet, Ar-CH), 7.29-7.31 (2H, doublet, Ar-CH), 7.43-7.45 (1H, multiplet, Ar-CH), 7.44-7.48 (1H, triplet, Ar-CH), 7.69-7.71 (1H, doublet, Ar-CH), 7.85-7.87 (1H, doublet, Ar-CH), 8.42 (1H, singlet, Ar-CH), *MS: (m/z)* = 369.7

NISO-6 *IR (cm^{-1}, KBr)*: 2899.25 (asym. C-H str.), 2845.55 (sym. C-H str.), 1456.27 (asym. C-H def.), 1423.33 (sym. C-H def.), 3020.78 (sym. aromatic C-H str.), 1522.65 (aromatic C=C str.), 1664.42 (C=N str.), 1300.07 (C-N str.), 812.62 (N-O str.), 1247.97 (asym. C-O-C str.), 1026.26 (sym. C-O-C str.), 1182.79 (C-F str.), 745.35 (C-Cl str.), *^{1}H NMR (DMSO-d_6) δ(ppm)*: 6.84-6.86 (2H, doublet, Ar-CH), 7.11 (1H, singlet, Ar-CH), 7.34-7.36 (2H, doublet, Ar-CH), 7.45-7.47 (1H, triplet, Ar-CH), 7.70-7.72 (1H, doublet, Ar-CH), 7.84-7.86 (1H, doublet, Ar-CH), 8.40 (1H, singlet, Ar-CH), 9.10 (1H, singlet, -OH), *MS: (m/z)* = 340.7

NISO-7 *IR (cm^{-1}, KBr)*: 2899.24 (asym. C-H str.), 2845.18 (sym. C-H str.), 1456.22 (asym. C-H def.), 1423.42 (sym. C-H def.), 3020.62 (sym. aromatic C-H str.), 1528.54 (aromatic C=C str.), 1664.22 (C=N str.), 1308.98 (C-N str.), 812.62 (N-O str.), 1247.67 (asym. C-O-C str.), 1026.17

(sym. C-O-C str.), 1182.16 (C-F str.), 745.35 (C-Cl str.), *^{1}H NMR (DMSO-d$_6$) δ(ppm)*: 1.21 (3H, singlet, -CH$_3$), 6.81-6.84 (2H, doublet, Ar-CH), 7.18 (1H, singlet, Ar-CH), 7.30-7.33 (2H, doublet, Ar-CH), 7.40-7.44 (1H, triplet, Ar-CH), 7.68-7.72 (1H, doublet, Ar-CH), 7.84-7.86 (1H, doublet, Ar-CH), 8.40 (1H, singlet, Ar-CH), *MS: (m/z)* = 338.8

NISO-8 *IR (cm^{-1}, KBr)*: 2899.20 (asym. C-H str.), 2845.39 (sym. C-H str.), 1456.21 (asym. C-H def.), 1423.46 (sym. C-H def.), 3020.67 (sym. aromatic C-H str.), 1531.54 (aromatic C=C str.), 1664.72 (C=N str.), 1333.61 (C-N str.), 833.18 (N-O str.), 1247.97 (asym. C-O-C str.), 1026.29 (sym. C-O-C str.), 1182.74 (C-F str.), 710.21 (C-Cl str.), *^{1}H NMR (DMSO-d$_6$) δ(ppm)*: 6.80-6.83 (2H, doublet, Ar-CH), 7.13 (1H, singlet, Ar-CH), 7.31-7.34 (2H, doublet, Ar-CH), 7.42-7.46 (1H, triplet, Ar-CH), 7.68-7.71 (1H, doublet, Ar-CH), 7.80-7.83 (1H, doublet, Ar-CH), 8.45 (1H, singlet, Ar-CH), *MS: (m/z)* = 359.2

NISO-9 *IR (cm^{-1}, KBr)*: 2899.24 (asym. C-H str.), 2845.17 (sym. C-H str.), 1456.54 (asym. C-H def.), 1423.71 (sym. C-H def.), 3020.53 (sym. aromatic C-H str.), 1514.38 (aromatic C=C str.), 1664.67 (C=N str.), 1298.75 (C-N str.), 841.21 (N-O str.), 1247.23 (asym. C-O-C str.), 1026.28 (sym. C-O-C str.), 1182.33 (C-F str.), 724.32 (C-Cl str.), *^{1}H NMR (DMSO-d$_6$) δ(ppm)*: 6.90-6.96 (2H, doublet, Ar-CH), 7.23-7.25 (2H, multiplet, Ar-CH), 7.43-7.45 (1H, triplet, Ar-CH), 7.68-7.70 (1H, doublet, Ar-CH), 7.87-7.89 (1H, doublet, Ar-CH), 8.46 (1H, singlet, Ar-CH), 9.17 (1H, singlet, -OH), *MS: (m/z)* = 340.7

NISO-10 *IR (cm^{-1}, KBr)*: 2899.29 (asym. C-H str.), 2845.99 (sym. C-H str.), 1456.14 (asym. C-H def.), 1423.16 (sym. C-H def.), 3020.73 (sym. aromatic C-H str.), 1501.52 (aromatic C=C str.), 1664.56 (C=N str.), 1300.26 (C-N str.), 833.28 (N-O str.), 1247.57 (asym. C-O-C str.), 1026.46 (sym. C-O-C str.), 1182.36 (C-F str.), 732.52 (C-Cl str.), *^{1}H NMR (DMSO-d$_6$) δ(ppm)*: 6.94-6.96 (2H, doublet, Ar-CH), 7.45-7.47 (3H, multiplet, Ar-CH), 7.44-7.48 (1H, triplet, Ar-CH), 7.62-7.65 (1H, doublet, Ar-CH), 7.83-7.85 (1H, doublet, Ar-CH), 8.44 (1H, singlet, Ar-CH), *MS: (m/z)* = 324.7
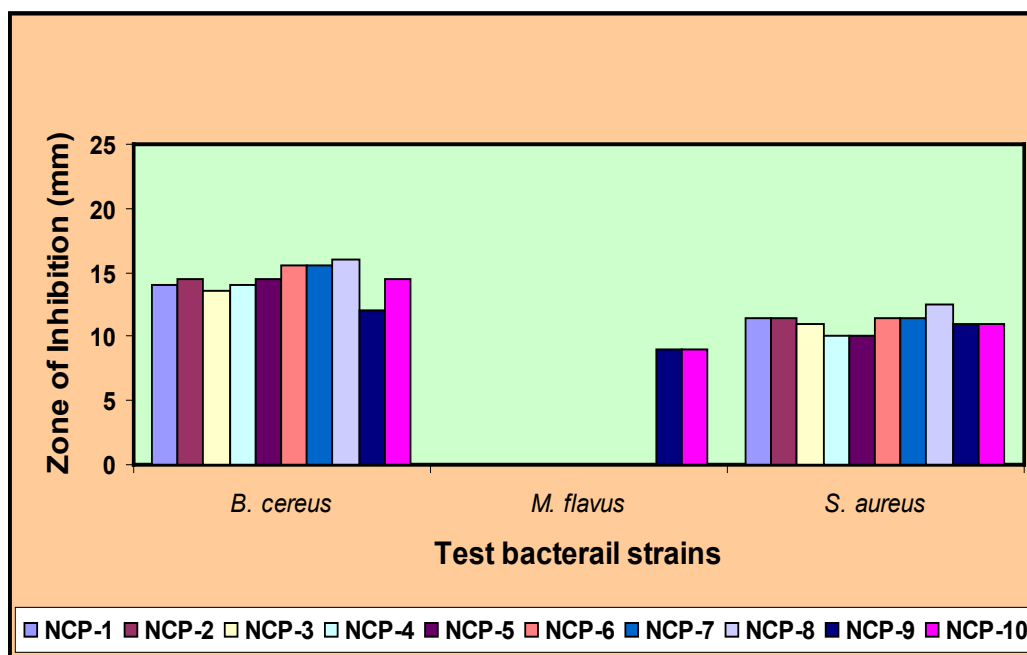
Antibacterial activity

Cyanopyridines:

Fig. 3 showed inhibition against Gram positive bacteria in DMSO. It is observed that against *B. cereus* except NCP-4, other compounds exhibited inhibition and is maximum for NCP-8 followed by NCP-6. There is no inhibition observed by any compounds against *M. flavus* and *S. aureus*.
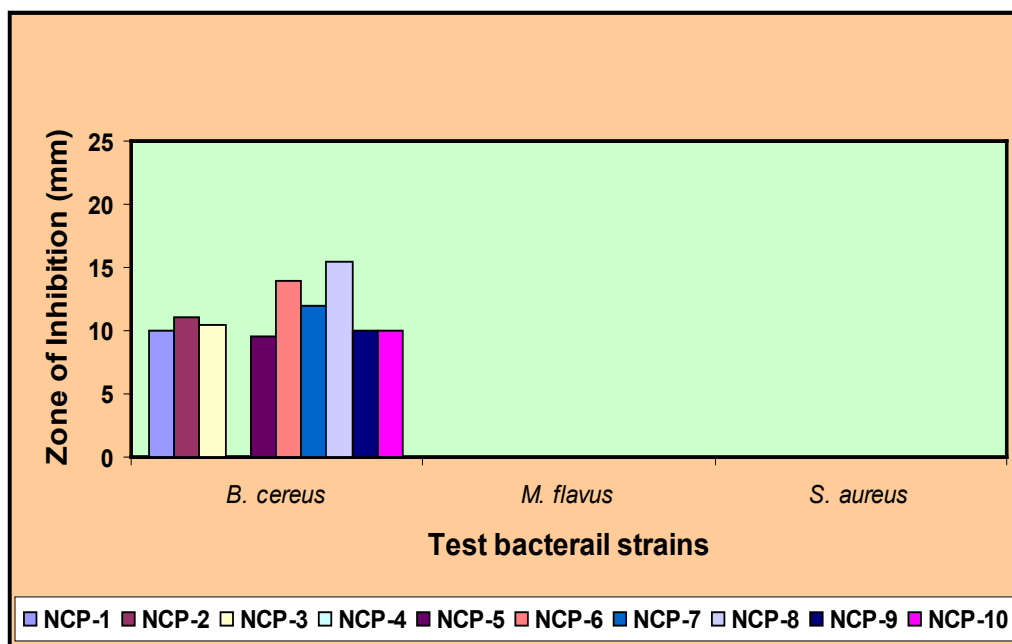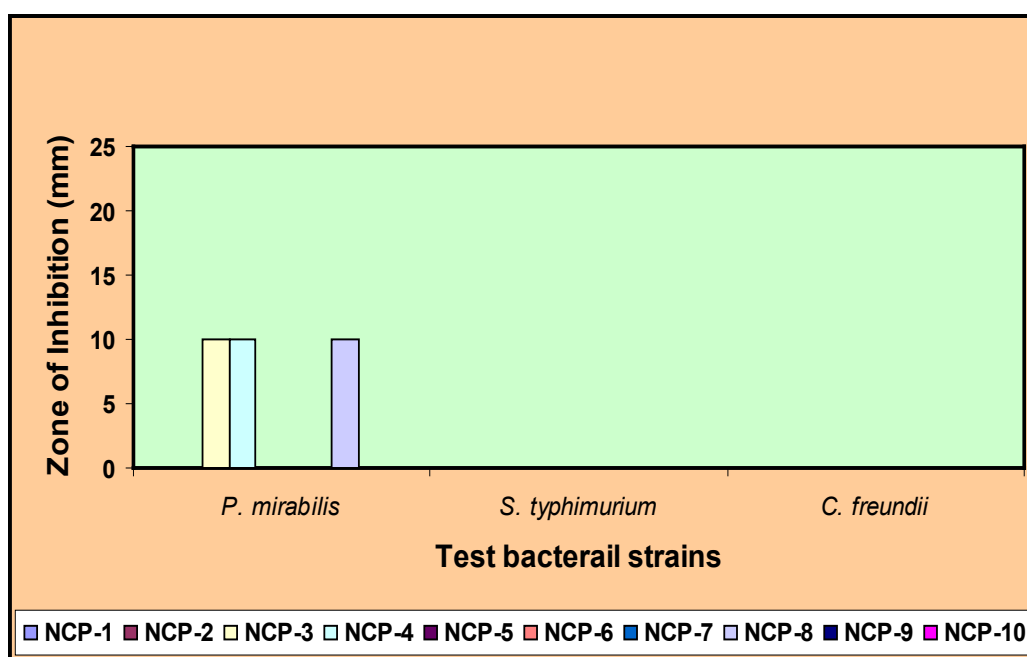

Figure 3. Antibacterial activity of cyanopyridines against Gram positive bacteria in DMSO.

However, in DMF, all the compounds exhibited inhibition against *B. cereus* and *S. aureus* as shown in Fig. 4. Further, maximum activity is for NCP-8. Against *M. flavus*, only NCP-9 and NCP-10 exhibited inhibition and inhibition is same for both the compounds.
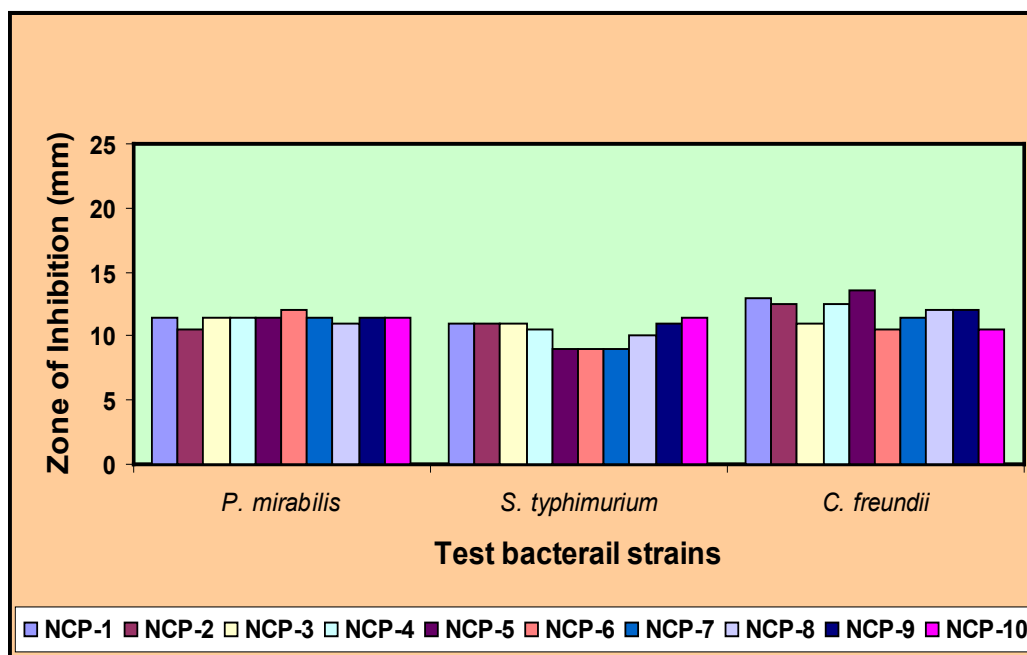

Figure 4. Antibacterial activity of cyanopyridines against Gram positive bacteria in DMF.

In case of Gram negative bacteria, Figs. 5 and 6 show zone of inhibition in DMSO and DMF respectively.

Figure 5. Antibacterial activity of cyanopyridines against Gram negative bacteria in DMSO.
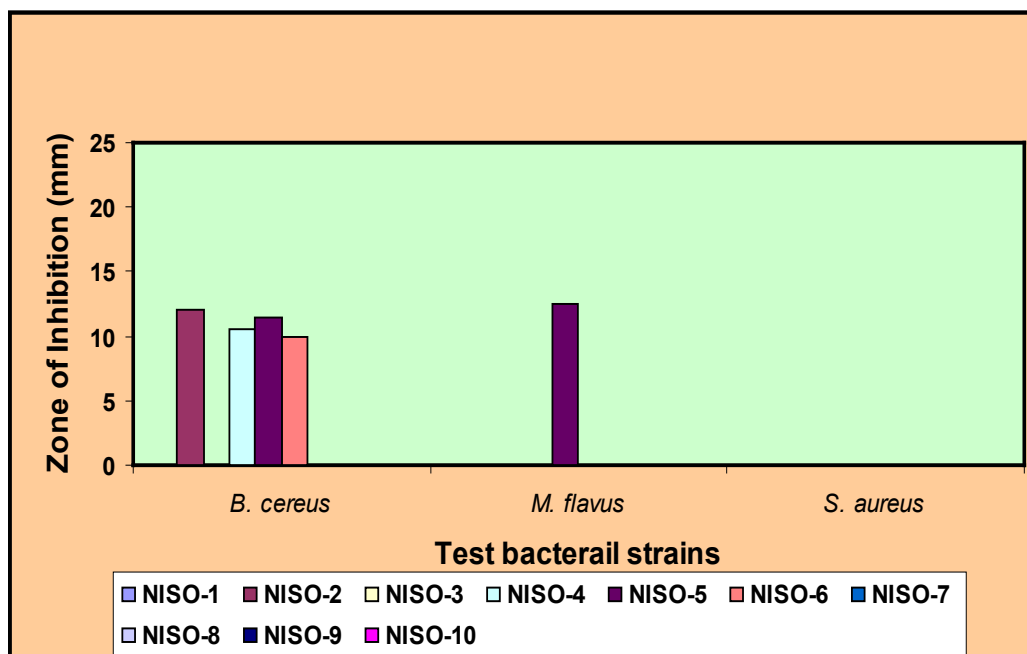
Figure 6. Antibacterial activity of cyanopyridines against Gram negative bacteria in DMF.

In DMSO, the studied compounds do not exhibit any activity against *S. typhimurium* and *C. freundii*. However, only NCP-3, NCP-4 and NCP-8 showed same inhibition for *P. mirabilis*. In DMF, all the compounds showed activity against all the three gram negative bacteria. For *P. mirabilis*, inhibition is almost similar for all the compounds. For *S. typhimurium*, maximum is for NCP-10 and minimum for NCP-5, NCP-6 and NCP-7.
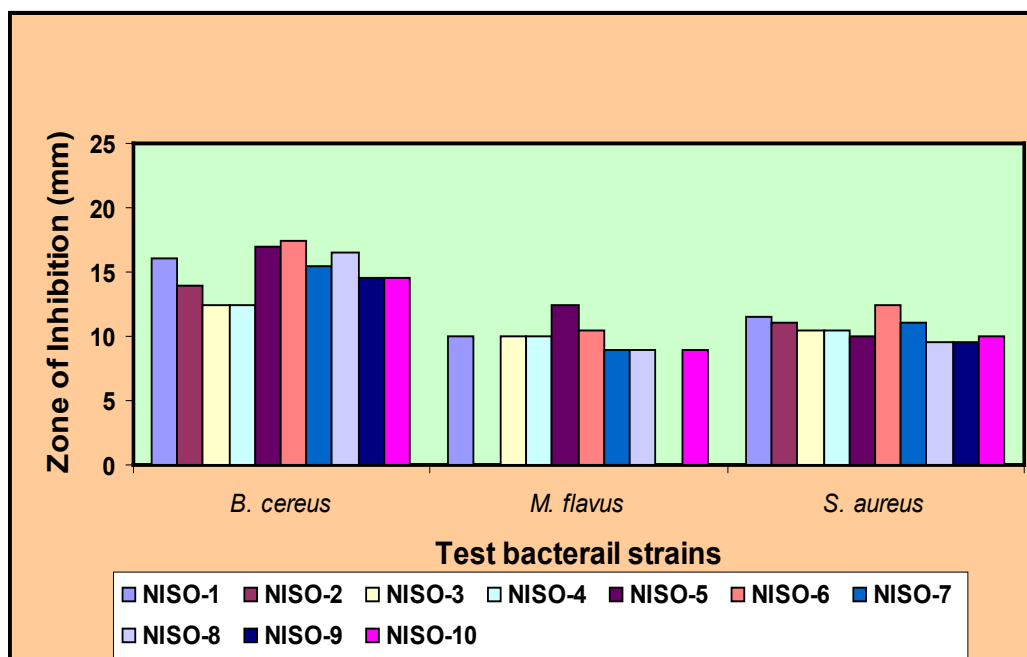
Isoxazoles

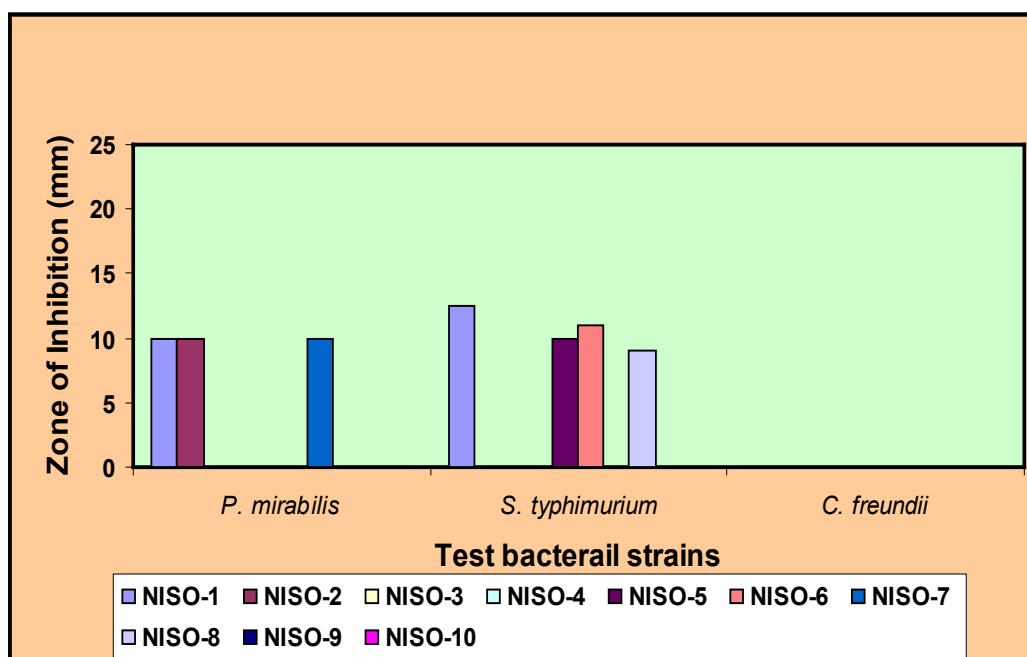Figs. 7 and 8 show inhibition against Gram positive bacteria in DMSO and DMF respectively.

Figure 7. Antibacterial activity of isoxazoles against Gram positive bacteria in DMSO.

In DMSO, for *B. cereus*, only NISO-2, NISO-4, NISO-5 and NISO-6 showed inhibition. Other compounds had no effect at all. In DMF, against *B. cereus*, all compounds exhibited inhibition and maximum inhibition is for NISO-6.
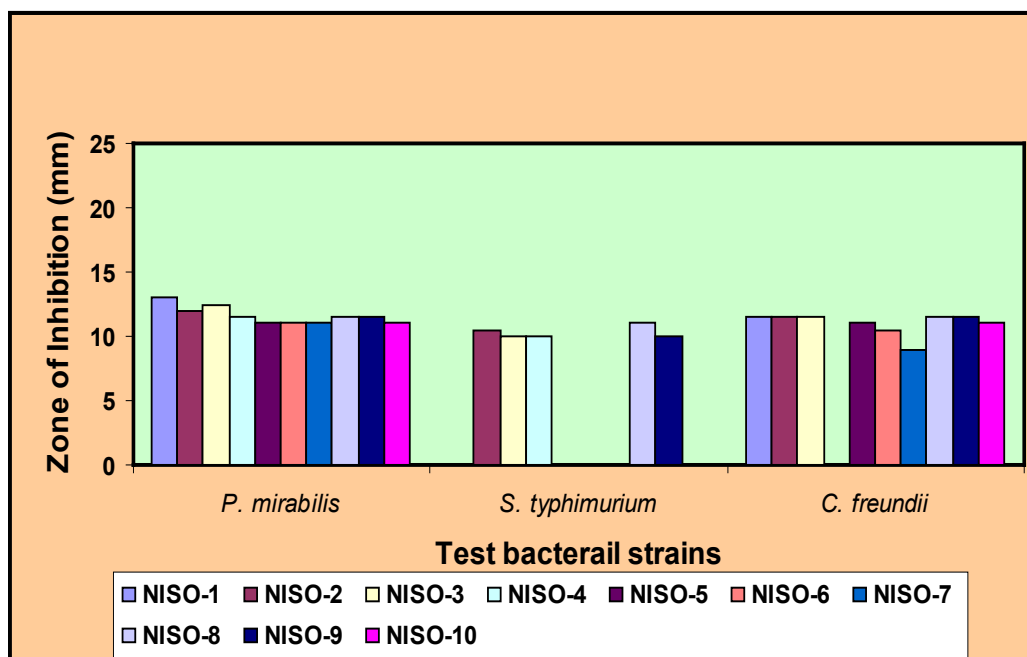
Figure 8. Antibacterial activity of isoxazoles against Gram positive bacteria in DMF.

Against Gram negative bacteria, zone of inhibition in DMSO and DMF are shown in Figs. 9 and 10 respectively.

Figure 9. Antibacterial activity of isoxazoles against Gram negative bacteria in DMSO.

In DMSO, against *P. mirabilis* only NISO-1, NISO-2 and NISO-7 showed inhibition which is of same magnitude. Others compounds showed no inhibition at all. For *S. typhimurium* NISO-1, NISO-5, NISO-6 and NISO-8 showed inhibition. Other compounds had no effect on this bacterium. Maximum inhibition is for NISO-1 and minimum for NISO-8. No inhibition is observed against *C. freundii* by any compounds.

Figure 10. Antibacterial activity of isoxazoles against Gram negative bacteria in DMF.

In DMF, all compounds showed almost similar inhibition against *P. mirabilis*. Against *S. typhimurium*, NISO-8 show maximum activity followed by NISO-2 whereas NISO-3, NISO-4 and NISO-9 show similar minimum activity while NISO-1, NISO-5, NISO-6, NISO-7 and NISO-10 shows no activity. Against *C. freundii*, all the compounds show almost similar average activity except NISO-4. Thus, all substitutions are effective for *C. freundii* except NISO-4.

Discussion

Cyanopyridines:

The results indicate that inhibition depends on three S: solvent, strain and structure. All the compounds have the same central moiety but different substitutions as given in Table 1. Comparison of inhibition against Gram positive bacteria of studied compounds in DMF and DMSO solvents shows that DMF is good solvent.

In DMSO, against *B. cereus* except NCP-4, other compounds exhibited inhibition. Table 1 shows that NCP-4 contains nitro group at para position. Thus, presence of nitro group could not affect this bacterial strain. The compound NCP-8 containing chloro group at para position is most effective. Against *M. flavus* and *S. aureus*, not a single compound is effective.

However, in DMF, again NCP-8 containing chloro group at para position exhibited maximum inhibition against *B. cereus* and *S. aureus*. Against *M. flavus*, hydroxyl group at ortho position (as in NCP-9) and only hydrogen (as in NCP-10) exhibited inhibition to the same compound. Other substitutions had no effect at all.

Thus, overall p-chloro substitution (as in NCP-8) is most effective against studied Gram positive bacteria.

In case of Gram negative bacteria, **i**n DMSO, against *S. typhimurium* and *C. freundii*, studied compounds had no effect. However, for *P. mirabilis* only NCP-3, NCP-4 and NCP-8 containing bromo, nitro and chloro substitutions at para position respectively showed inhibition to the same extent.

In DMF, all the substitutions are found to be effective against all the three gram negative bacteria. The compound having no substitution i.e., NCP-10 is most effective against *S. typhimurium* whereas nitro group at meta position (as in NCP-5), hydroxyl group at para position (as in NCP6) and methyl group at para position (as in NCP-7) are least effective. For *P. mirabilis*, all substitutions are almost equally effective. However, when there is no substitution group present

(as in NCP-10), it is most effective for this bacteria. Against *C. freundii*, nitro group at meta position (as in NCP-5) is most effective whereas maximum inhibition is for NCP-5.

Overall, DMF is good solvent for Gram negative bacteria.

Isoxazoles:

Against Gram positive bacteria in DMSO, for *B. cereus*, amino group at ortho position is most effective whereas against *M. flavus*, only nitro group at meta position had effect. However, against *S. aureus*, all the substitution in studied compounds had no effect on studied bacterial strains. Thus, *S. aureus* is most resistant bacteria in DMSO.

In DMF, against *B. cereus*, all the substitution in studied compounds had effect and effect is maximum for compound containing hydroxyl group at para position (i.e., NISO-6). The bromo and nitro groups at para position had little effect.

Against *M. flavus*, nitro group at meta position (as in NISO-5) causes maximum inhibition which is followed by similar inhibition of methoxy (in NISO-1), bromo (in NISO-3) and nitro group (in NISO-4) at para position. The inhibition due to other groups is comparatively less. However, amino group at para position (as in NISO-2) and hydroxyl group at ortho position (as in NISO-9) had no effect at all. Thus, in this case m-nitro group is most effective. For *S. aureus*, again all compounds showed inhibition and hydroxy group at para position is most effective. The chloro group at para position and hydroxy group at ortho position had minimum effect.

Thus in DMF, overall compounds containing nitro group at meta position and hydroxy group at para position are most effective.

Against Gram negative bacteria, in DMSO only methoxy, amino and methyl groups at para positions are effective against *P. mirabilis*. Others substitutions showed no inhibition at all. For *S. typhimurium,* methoxy, nitro, hydroxyl and chloro groups at para positions had effect. Other compounds had no effect on this bacterium. Maximum inhibition is for NISO-1 containing methoxy group and minimum for NISO-8 having chloro group. Thus, for *S. typhimurium,* p-methoxy substitution is most effective. No inhibition is observed against *C. freundii* by any compounds. Thus, in DMSO, *C. freundii* is most resistant bacteria.

In DMF, all compounds showed almost similar inhibition against *P. mirabilis*. Against *S. typhimurium*, chloro group at para position (as in NISO-8) causes maximum inhibition which is followed by amino group at para position (as in NISO-2). The bromo (as in NISO-3), nitro (as in NISO-4) and hydroxyl at ortho position (as in NISO-9) had similar minimum inhibition while other groups had no effect. Against *C. freundii*, all substitutions are effective for *C. freundii* except p-nitro (as in NISO-4). Thus, in DMF, *S. typhimurium* is most resistant bacteria.

Conclusions

Cyanopyridine and isoxazole derivatives were synthesized by using Vilsmeier-Haack reagent. The structures of synthesized compounds were supported by spectroscopic techniques. The antibacterial screening of these compounds shows that inhibition depends on solvent, bacterial strain and compound structure. For both Cyanopyridine and isoxazole derivatives, DMF is good solvent. Against different bacterial strains, different compounds have different effect. However, overall, S. aureus, M. flavus and C. freundii are the most resistant bacteria in the studied solvents for these synthesized compounds.

Conflict of Interest

Authors have no conflict of interest.

Acknowledgement

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