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# SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF SOME THIENO PYRIDINE DERIVATIVES

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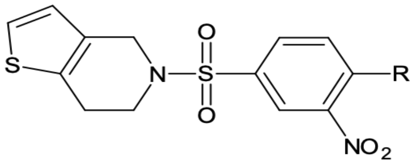
## ABSTRACT

In the present work biodynamic activities of pyridine and its derivatives have been observed intensively. Pyridine derivatives have also been synthesized by nucleophilic substitution reactions. The constitution of the synthesized products have been characterized by using Elemental analysis, IR, $^{1}$H NMR, $^{13}$C and MS. Purity of all compounds have been observed by TLC. All the newly synthesized compounds were screened for antibacterial and antifungal activities (MIC) in vitro by both dilution method with two Gram positive bacteria (S. Aureus MTCC 96 and S. pyogenus MTCC 442), two Gram negative bacteria (E. Coli MTCC 443 and P. aeruginosa MTCC 1688) and fungi A. niger MTCC 282.

**KEYWORDS:** Synthesis, Characterization, Biological Evaluation, Pyridine Derivatives.

## INTRODUCTION

Keeping in view of wide spectrum biodynamic activities[1-45] of pyridine and with a view to have therapeutic agents, the synthesis of 5 - (4 - Substituted phenoxy - 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 -Tetrahydrothieno[3, 2 - C] Pyridine (2a-d) have been by the nucleophilic substitution of the chloro atom of 5 - (4 - Choro - 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 -Tetrahydrothieno[3, 2 - C] Pyridine with different substituted phenols.



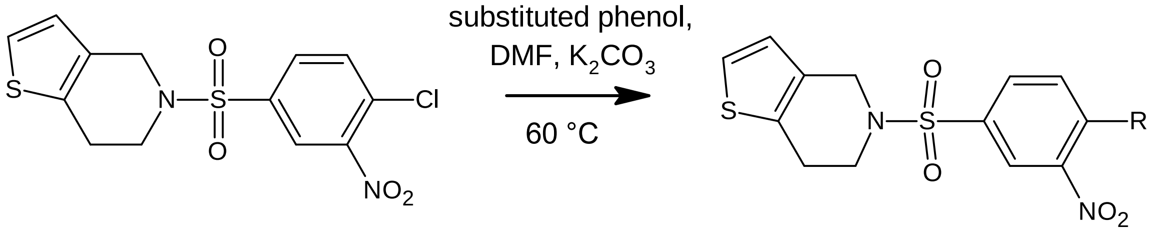
**(2a-d)**

### Substituted phenols

The constitution of the synthesized products (2a-d) have been characterized by using elemental analysis, infrared and $^1$H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the newly synthesized compounds 3a-d were screened for antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method[64] with two Gram positive bacteria (S. Aureus MTCC 96 and S. pyogenus MTCC 442), two Gram-negative bacteria (E. Coli MTCC 443 and P. aeruginosa MTCC 1688) and fungi A. niger MTCC 282.

### Reaction scheme



**Scaffold-A** **(2a-d),**

**R=Substituted Phenols**

## EXPERIMENTAL

Preparation, characterization and antimicrobial evaluation of 5 –[4- (4 - Substituted phenoxy - 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 –Tetrahydrothieno [3, 2 - C] Pyridine.

### [A] Synthesis of scaffold-A

A mixture of 4, 5, 6, 7 –Tetra hydro thieno [3, 2 – c] pyridine hydrochloride (1.0 mole) in THF and $K_2CO_3$ (3.0 mole) in RBF was added at 25 – 30 $^0$C for 10 – 15 min on magnetic stirrer. Then 4 – chloro 3 – nitro benzene sulfonyl chloride (1.1 mole) slowly added in reaction mixture with constant stirring for 15 mins at 10-15 $^0$C and then allowed to warm at room temperature. Reaction mixture was stirred at room temperature for 2 h. After completion of reaction, the reaction mixture was distilled out. Water was added to the obtained residue and then again it stirred for 1 h 30 min. Solid was filtered out, triturated with ethanol and filtered to get pure product. The solid was dried under reduced pressure. Yield; 55.50 %, m.p. 230-232 $^0$C.

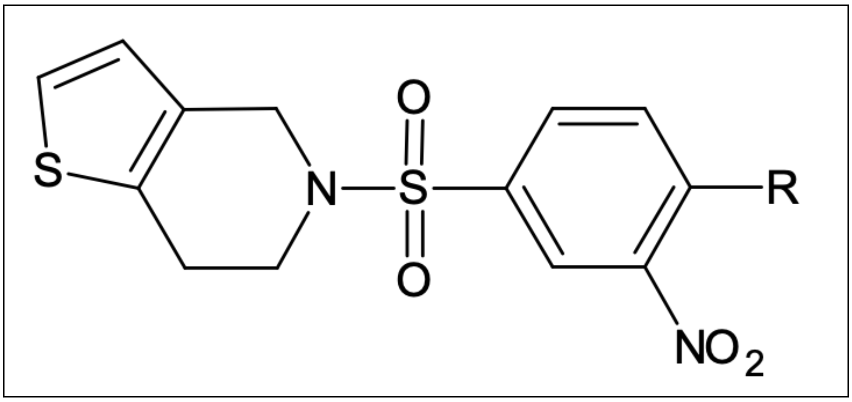
[B] Synthesis of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine.

To the stirred solution of scaffold-A (1 mole) in DMF was added $K_2CO_3$ and 4-methoxyphenol (2 mole) at 60 $^0$C for 4 hours with help of magnetic stirrer. The reaction mixture was stirred at reflux temperature for 3 hours at room temprature and the solvent was evaporated under reduced pressure. The pH of the mixture was more than 7, so add dil.HCl to reduced pH upto 6.5 and again stir reaction mixture for 2 hrs. at room temperature. Water was added to the obtained residue and stirred again for 1 hour at room temperature. Solid was filtered out, triturated with ethanol and filtered to get product. The solid was dried under reduced pressure. Yield; 65%, m.p 185$^0$C. Elemental analysis; Calculated; C (53.80%), H (4.06%), N (6.27%), O (21.50%), S(14.36%). Elemental analysis; Found; C (53.78%), H(4.05%), N (6.29%), O (21.48%), S(14.35%).

Similarly, other compounds (2a-d) were synthesized by above mentioned process (B) from Scaffold-A using different substituted phenols. The physical data are recorded in Table-1.

**Physical Properties**

Table 1: Physical constants of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine.



**Spectral studies**

Spectral studies like IR, $^1$H NMR, $^{13}$C NMR, Mass Spectra (MS) and HRMS have been carried out for all the Pyridine derivatives.

**Antibacterial and antifungal activity**

All the newly synthesized compounds 1a-d were screened for antibacterial and antifungal ac-

tivity (MIC) *in vitro* by broth dilution method with two Gram positive bacteria (S. aureus MTCC 96 and S. pyogenus MTCC 442), two Gram-negative bacteria (E. coli MTCC 443 and P. aeruginosa MTCC 1688) and fungi A. niger MTCC 282 taking ampicillin, chloramphenicol, nystatin and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The observed data of synthesized compounds are given in Table 2.
All the glass apparatus used were sterilized before use.

## RESULTS AND DISCUSSION

**Table 1: Physical Data Of Compound 2a-D.**

| Comp. | Substitution R | Molecular Formula/Weight | M. P. $^0$C | Yeild % | % Composition Calculated/Found | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| 2a | 4-methoxy phenol | $C_{20}H_{18}N_2S_2O_6$<br>446 | 185 | 65 | 53.80<br>53.78 | 4.06<br>4.05 | 6.27<br>6.29 |
| 2b | 4-hydroxy benzonitrile | $C_{20}H_{15}N_3S_2O_5$<br>441 | 193 | 65 | 54.41<br>54.40 | 3.42<br>3.39 | 9.52<br>6.50 |
| 2c | 2-chloro phenol | $C_{19}H_{15}ClN_2S_2O_5$<br>451 | 172 | 68 | 50.61<br>50.59 | 3.35<br>3.33 | 6.21<br>6.19 |
| 2d | 4-fluoro phenol | $C_{19}H_{15}FN_2S_2O_5$<br>434 | 178 | 68 | 52.53<br>52.51 | 3.48<br>3.46 | 6.45<br>6.43 |

### Spectral studies

IR Spectral study of 5-{4-[(4-methoxyphenyl)sulfanyl]-3-nitrobenzene-1-sulfonyl}-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine **(2-a).**

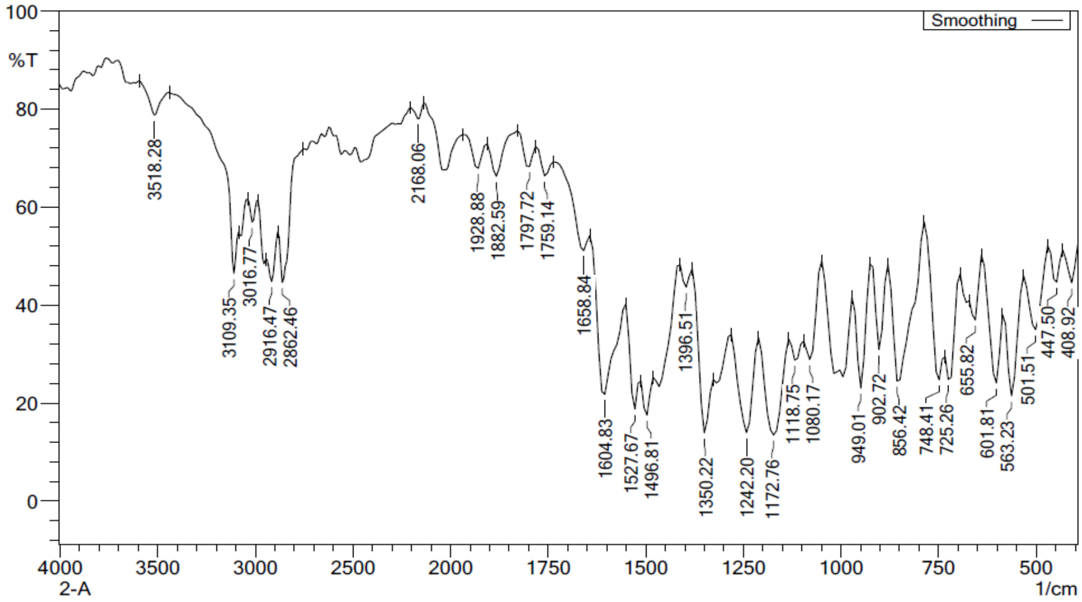


**Figure 1**

**Table 2: IR data of compound 2a.**

| Vibrating Frequency ($cm^{-1}$) | Functional group |
|---|---|
| 3109, 3016 | Aromatic C-H stretching |
| 2916, 2862 | Aliphatic C-H Stretching |
| 1604 | C=C stretching |
| 1527 | $NO_2$ Stretching |
| 1350, 1242 | S=O stretching |

$^1$H NMR Spectral study of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine (2-a).

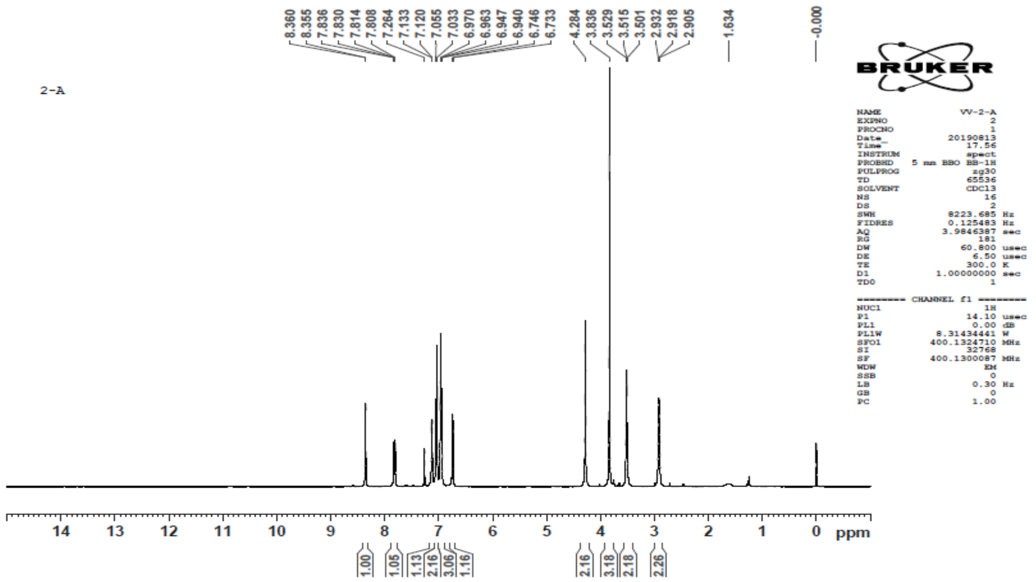
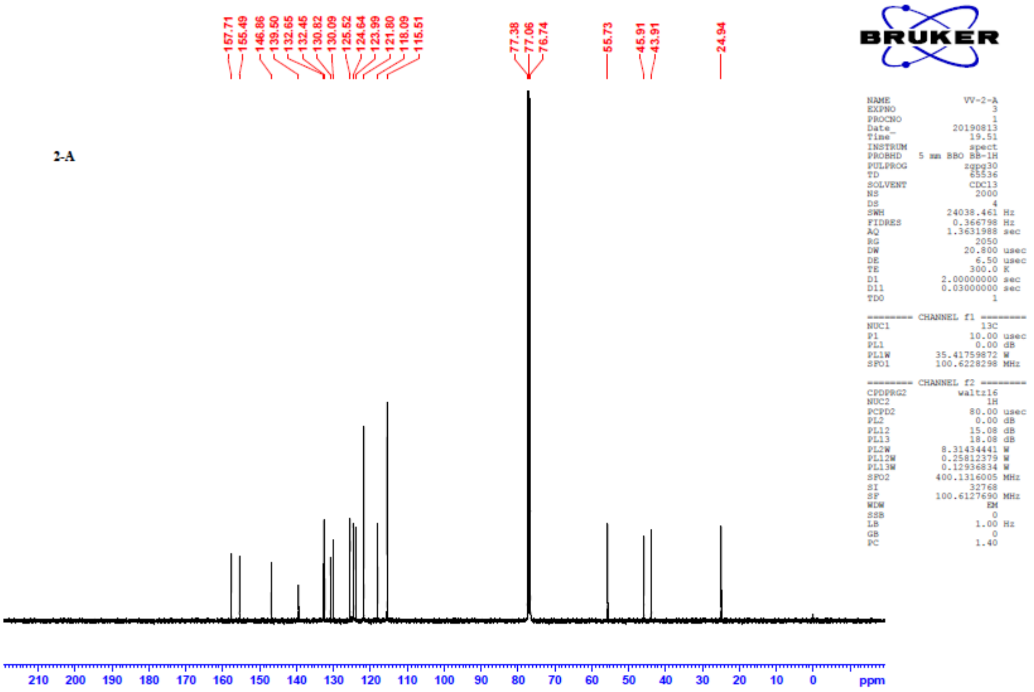


**Figure 2.**

**Table 3: $^1$H NMR data of compound 2a.**

| Chemical Shift | No of Proton | Multiplicity and J value in Hz |
|---|---|---|
| 8.360-8.355 | 1 | Doublet J=2 |
| 7.836-7.808 | 1 | Doublet of doublet J=2.4, 8.8 |
| 7.133-7.120 | 1 | Doublet J=5.2 |
| 7.055-6.940 | 4 | Multiplet |
| 6.746-6.733 | 1 | Doublet J=5.2 |
| 4.225 | 2 | Singlet |
| 3.466-3.437 | 2 | Triplet J=5.6, 11.6 |
| 2.934-2.907 | 2 | Triplet J=5.6, 10. |
| 2.041-2.026 | 4 | Multiplet J=6 |

$^{13}$C NMR Spectral study of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine (2-a).



```
NAME            VV-2-A
EXPNO                3
PROCNO               1
Date_         20190813
Time             19.51
INSTRUM          spect
PROBHD   5 mm BBO BB-1H
PULPROG        zgpg30
TD               65536
SOLVENT          CDCl3
NS                2000
DS                   4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ           1.3631988 sec
RG                2050
DW              20.800 usec
DE                6.50 usec
TE               300.0 K
D1          2.00000000 sec
D11         0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1               13C
P1               10.00 usec
PL1               0.00 dB
PL1W       35.41759872 W
SFO1       100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2        waltz16
NUC2                1H
PCPD2            80.00 usec
PL2               0.00 dB
PL12             15.08 dB
PL13             18.08 dB
PL2W        8.31434441 W
PL12W       0.25812379 W
PL13W       0.12936834 W
SFO2       400.1316005 MHz
SI               32768
SF         100.6127690 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

**Figure 3.**

**Table 4: $^{13}$CMR data of compound 2a.**

| δ Value | No. Of Carbon |
|---|---|
| 24.94 | 1C |
| 43.91 | 1C |
| 45.91 | 1C |
| 55.73 | 1C |
| 115.51 | 1C |
| 118.09 | 1C |
| 121.80 | 1C |
| 123.99 | 1C |
| 124.64 | 1C |
| 125.52 | 2C |
| 130.09 | 1C |
| 130.82 | 1C |
| 132.45 | 1C |
| 132.65 | 2C |
| 139.50 | 1C |
| 146.86 | 1C |
| 155.49 | 1C |
| 157.71 | 1C |

Mass spectroscopic study of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine (2-a).

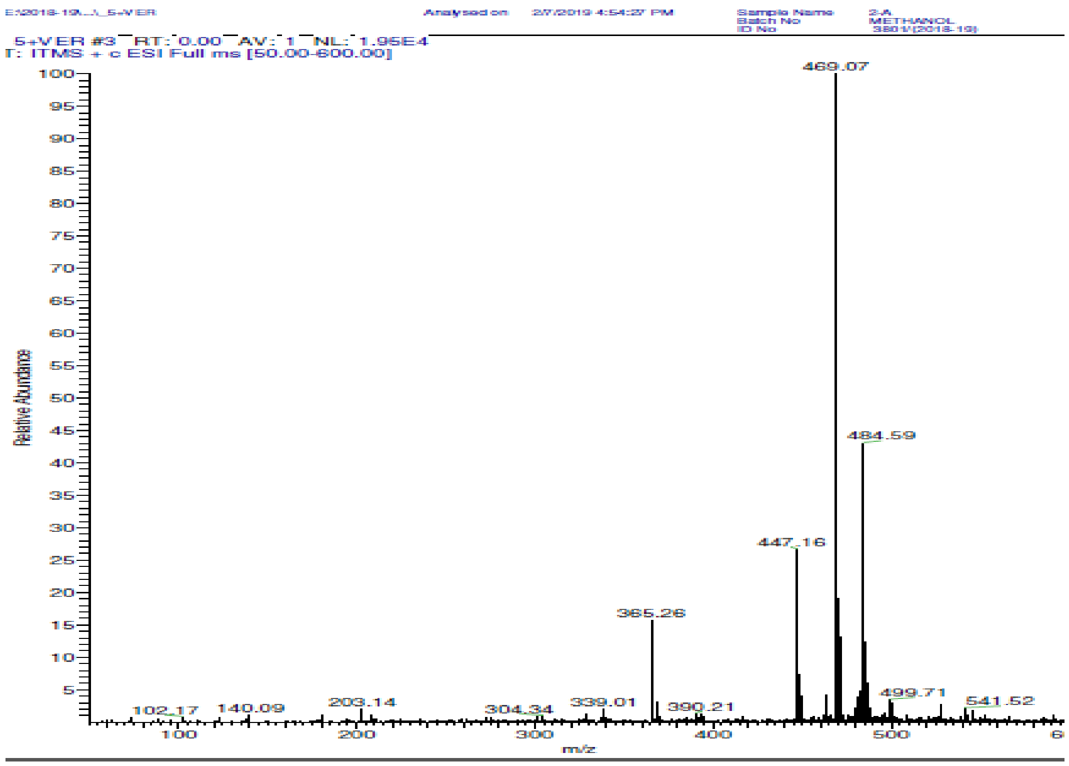


**Figure 4.**

## Mass in m/z

Molecular ion peak was observed at 447.16 [M + 1]

## ANTIMICROBIAL ACTIVITY

The MICs of all the synthesized compounds was carried out by broth dilution method. Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to $10^8$ CFU [Colony Forming Unit] per milliliter by comparing the turbidity. Dimethyl sulfoxide (DMSO) was used as diluent to get desired concentration of drugs to test on standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 $^{o}$C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) was subcultured and incubated overnight at 37 $^{o}$C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted to 2000 µg/ml concentration, as a stock solution. In primary screening 1000, 500 and 250 µg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50 and 25 µg/ml concentrations. The highest dilution showing at least 99% inhibition is taken as MIC. The activities of all the synthesized compounds are recorded in Table 1.

**Table 1: Antimicrobial activity of 5 - {4 - [(4 - methoxyphenyl) sulfanyl] - 3 - nitrobenzene - 1 - sulfonyl} - 4, 5, 6, 7 - tetrahydrothieno [3, 2 - *c]* pyridine.**

**Table 5 Antimicrobial activity of 5 –[4- (4 - Substituted phenoxy - 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 –Tetrahydrothieno [3, 2 - C] Pyridine.**

| **Comp. No.** | **Minimal bactericidal concentration (µg/ml)** | | | | **Minimal fungicidal Concentration (µg/ml)** |
|---|---|---|---|---|---|
| | **Gram-negative** | | **Gram-positive** | | |
| | **E. coli** | **P. aeruginosa** | **S. aureus** | **S. pyogenes** | **A. niger** |
| 2a | 150 | 100 | 100 | 200 | 250 |
| 2b | 200 | 200 | 200 | 150 | 1000 |
| 2c | 125 | 150 | 150 | 100 | 500 |
| 2d | 150 | 125 | 100 | 150 | 1000 |
| Ampicillin | 100 | -- | 250 | 100 | -- |
| Chloramphenicol | 50 | 50 | 50 | 50 | -- |
| Nystatin | -- | -- | -- | -- | 100 |
| Griseofulvin | -- | -- | -- | -- | 100 |

## ANTIMICROBIAL EVALUATION

In the series 2a-d, compound 2c and 2d found comparative inhibitory activity against E.coli. with respect to ampicillin. Compound 2c and 2d having electron withdrawing fluorine and chlorine group at ortho and para position respectively found two fold lessactive against E. coli. with respect to ampicillin. Compound 2b having electron withdrawing group cyno at para position of the phenyl ring found more than two fold less active against E. coli. with respect to ampicillin. Compound 2a having electron donaing methoxy group at para position of the phenyl ring respectively found almost two fold less potent against E. coli. with respect to ampicillin.

For p. aeruginosa, compound 2a having electron donating methoxy group at para position found most reactive in the series, however, chloramphenicol was still two fold more potent than compound 2a. Compound 2b, 2c and 2d having electron withdrawing group cyno, chloro and fluoro at ortho or para position found to possess good inhibitory activity in the series, still almost two fold less active compare to chloramphenicol. Replacement of electron withdrawing group ortho to para position of the phenyl ring respectively found more active but still they found respectively less active against p.aeruginosa with respect to chloramphenicol.

For s.aureus, all the compound (2a-d) found more potent against s. aureus with respect to ampicillin. Compound 2d having electron withdrawing chlorine and fluorine at ortho or para position respectively found more than two fold more active against s. aureus with respect to ampicillin. Compound 2a having electron donating methoxy group at para position respectively found more than two fold more reactive against s. aureus with respect to ampicillin. Compound 2b having electron withdrawing cyno group at para position decrease the inhibition activity by almost two fold, however, still it found more potent against s. aureus with respect to ampicillin.

For s. pyogenes, compound 2c having electron withdrawing group at ortho position of the phenyl ring found equipotent against s. pyogenes with respect to ampicillin. All other compound in these series found less potent against s. pyogenes with respect to ampicillin. These finding suggested that phenols having electron withdrawing group at ortho position of the phenyl ring are better substituent in chloro nitro pyridine.

For fungi, compound 2a having electron donating methoxy group at para position of the phenyl ring found most potent against among the series (2a-d), however, found still more than two fold less potent against A. niger with respect to nystatin and griseofulvin.

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