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

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Abstract:

A novel series of thienopyridine derivatives, particularly 5–(4-morpholin-4-yl)-3-nitrobenzene-1-sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine, was synthesized and evaluated for antimicrobial activity. Structural characterization was carried out using spectral techniques, including IR, NMR, and mass spectrometry. The synthesized compounds were tested against a panel of Gram-positive and Gram-negative bacterial strains (*S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*) and fungal species (*A. niger*) using the broth dilution method to determine minimum inhibitory concentrations (MICs). Among the tested compounds, derivatives with piperazine and piperidine moieties demonstrated better activity profiles compared to morpholine analogs. The findings suggest the potential of these sulfonylated thienopyridines as promising antimicrobial agents with further optimization.

Keywords: Thienopyridine derivatives, Morpholine analog, antimicrobial activity, Broth dilution method, Minimum inhibitory concentration (MIC)

Introduction

The increasing resistance of pathogens to conventional antimicrobial agents has necessitated the search for novel therapeutic scaffolds with enhanced potency and specificity. Heterocyclic compounds, particularly thienopyridines, have attracted attention due to their diverse pharmacological properties, including antimicrobial, anticancer, and anti-inflammatory activities. In this context, the incorporation of sulfonyl and morpholine functional groups into the thienopyridine framework is a strategic approach to improve biological activity and drug-like properties. [1]

In the present study, we report the synthesis, characterization, and antimicrobial evaluation of a novel compound, 5–(4-morpholin-4-yl)-3-nitrobenzene-1-sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine, and its analogs. The biological screening was conducted using standard microbial strains, and the results provide insights into the structure-activity relationship (SAR) within this chemical series. This work aims to explore the therapeutic potential of these derivatives and contribute to the development of new antimicrobial agents. [2]

Materials and Method:

Preparation of 5 - (4 - Substituted amino) - 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 – Tetrahydro thieno [3, 2 - C] produced

products Elemental analysis, infrared, and 1H nuclear magnetic resonance spectroscopy, as well as mass spectrometry, have all been used to characterize pyridine. Thin layer chromatography was used to ensure that all chemicals were pure.

In broth dilution procedure 64, two gramme positive bacteria (Staphalococcus aureus, MTCC 96, and Staphalococcuspyogenus, MTCC 442), two gramme negative bacteria (E. Coli, MTCC 443, and P. aeruginosa, MTCC 1688), and fungi A. niger, MTCC 282, were utilised to test all newly synthesised compounds 1a-d for antimicrobial activity.

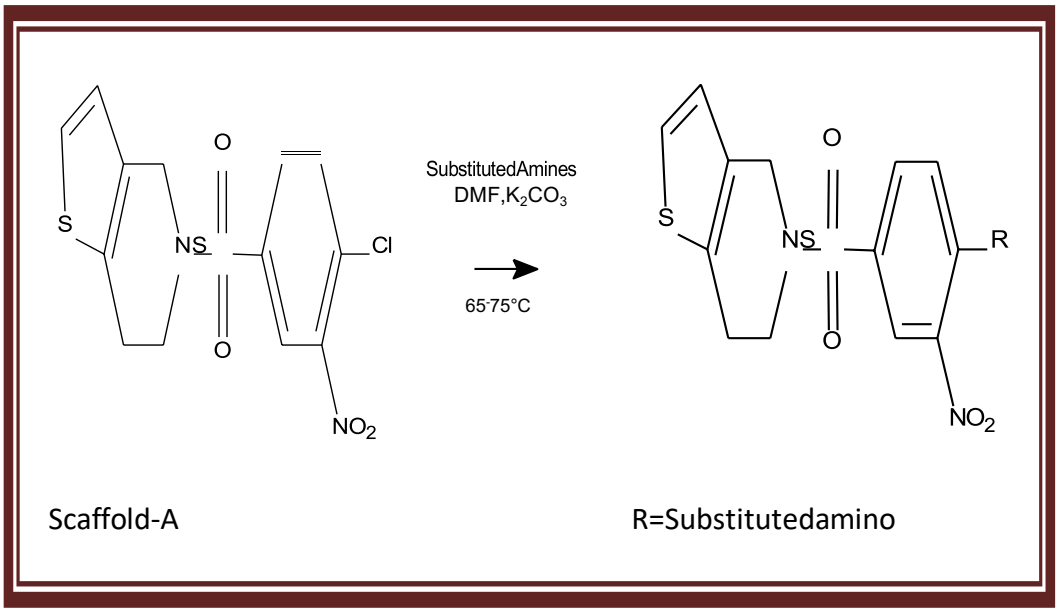


Figure 1 2ReactionSchemeof5–[4-(morpholin-4-yl)-3-nitrobenzene-1–sulfonyl]-4,5,6,7–tetrahydrothieno [3,2-c] pyridine

Synthesis of 5–[4-(morpholin-4-yl)-3-nitrobenzene-1–Sulfonyl]-4,5,6,7 Tetrahydrothieno[3,2-C] Pyridine. [3-4]

Transfer 1.0 mL (5-4-chloro-3-nitrobenzene-1-sulfonyl) -4,5,6,7-tetrahydrothieno [3,2-C] pyridine was mixed with DMF, K2CO3, and 2.0mole morpholin and allowedto sit at room temperature for 45 minutes. The reaction mixture was agitated at 75C for 3-4hours at reflux temperature before the solvent was evaporated under reduced pressure. Because the pH ofthe combination was higher than7.0, diluted HClwas added to lower the pH to 6.5 and the reaction mixture was stirred for another 2 hours at room temperature. Then, to the resulting residue, add Water and stir for 1 hour at room temperature. The solid was filtered away, then the mixture was triturated with ethanol and strained to obtain the product. The solid was dried under reduced pressure.Yield:68%,M.P 1850C. Elemental analysis of the compound Calculated amount of; H(4.67%), N (10.27%), O (19.52%), S(15.65%), C(49.86%), H(4.68%), N(10.26%), O(19.54%), S(15.66%) and similarly results found are

Table 1 Found yeild %of 5–[4-(morpholin-4-yl)-3-nitrobenzene -1– Sulfonyl]-4, 5, 6, 7–Tetrahydrothieno [3, 2-C] Pyridine.

Comp.	Substitution R	Molecular Formula/ Weight	M.P. 0C	Yeild %	% Composition Calculated/Found		
					C	H	N
1b	Morpholine	C17H19N3S2O5409	185	68	49.86 49.85	4.68 4.65	10.26 10.23

Antimicrobial Activity [5-8]

Protocol for Antimicrobial Activity

The antimicrobial activity of newly synthesized compounds — 5-(4-morpholin-4-yl)-3-nitrobenzene-1-sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine — was evaluated using the broth dilution method.

The following microbial strains were tested:

- Gram-positive bacteria: Staphylococcus aureus (MTCC 96), Streptococcus pyogenes (MTCC 442)
- Gram-negative bacteria: Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 1688)
- Fungus: Aspergillus niger (MTCC 282)

These standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

All glassware and apparatus used in the experiments were sterilized prior to use.

Medium Used

- Mueller-Hinton Broth (MHB): Used as the nutrient medium for bacteria.
- Sabouraud Dextrose Broth (SDB): Used for fungal growth.
- Inoculum Preparation
- A standardized inoculum of 10^8 CFU/mL was prepared by adjusting the turbidity of bacterial cultures to match a 0.5 McFarland standard.

Drug Preparation and Dilution

- All synthesized compounds were initially dissolved in Dimethyl Sulfoxide (DMSO).
- A stock solution of 2000 µg/mL was prepared.
- Serial dilutions were made to obtain concentrations of 1000, 500, 250 µg/mL for primary screening.
- Compounds showing promising results were further tested with additional dilutions: 200, 125, 100, 62.5, 50, and 25 µg/mL.

Procedure

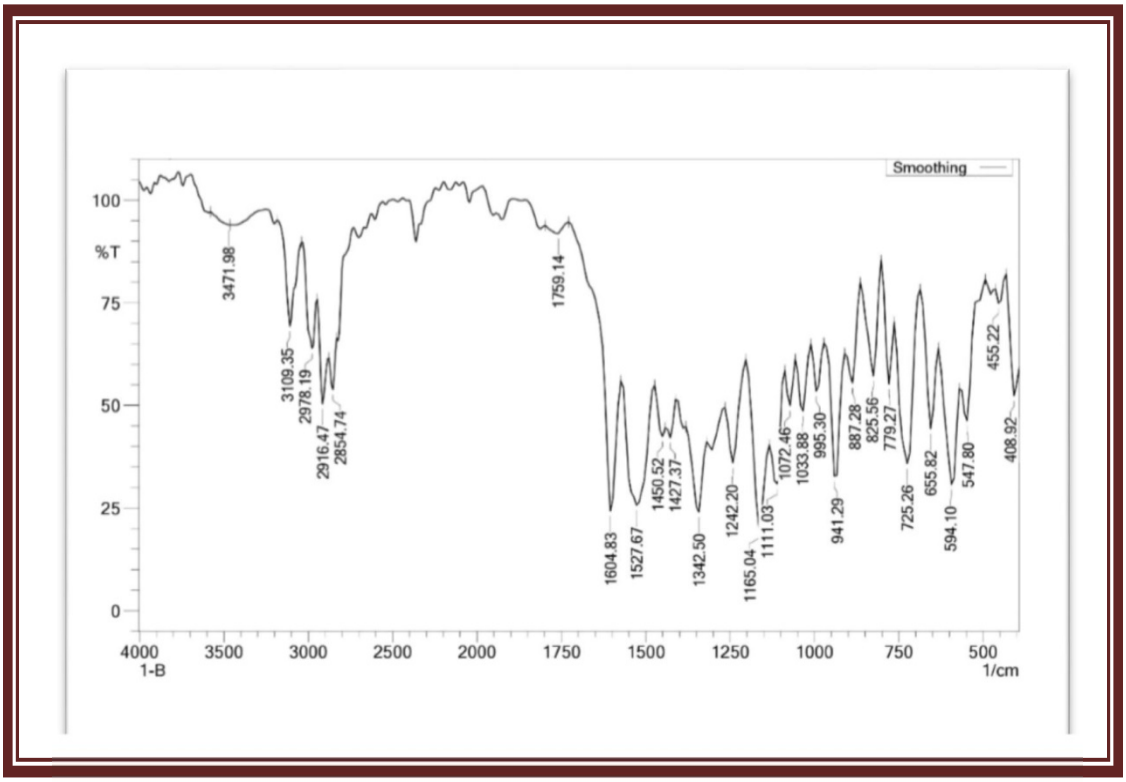
- Each test tube was inoculated with the standardized inoculum and the respective drug concentration.
- A control tube (no drug) was included to observe the normal growth of the organisms.
- The tubes were incubated overnight at 37°C.

Determination of MIC (Minimum Inhibitory Concentration)

- The MIC was defined as the lowest concentration of the compound that resulted in at least 99% inhibition of visible microbial growth compared to the control.
- Absence of visible turbidity was considered as no microbial growth.
- Confirmation was done through subculturing, where the bacteriostatic or bactericidal nature of the compound was inferred based on the number of colonies grown:
    - No growth = bactericidal activity

- Reduced or equal colonies compared to control = bacteriostatic or ineffective

IR Spectra of 5–[4-(morpholin-4-yl)- 3-nitrobenzene- 1 –Sulfonyl]- 4,5,6, Tetrahydrothieno [3,2-C] Pyridine

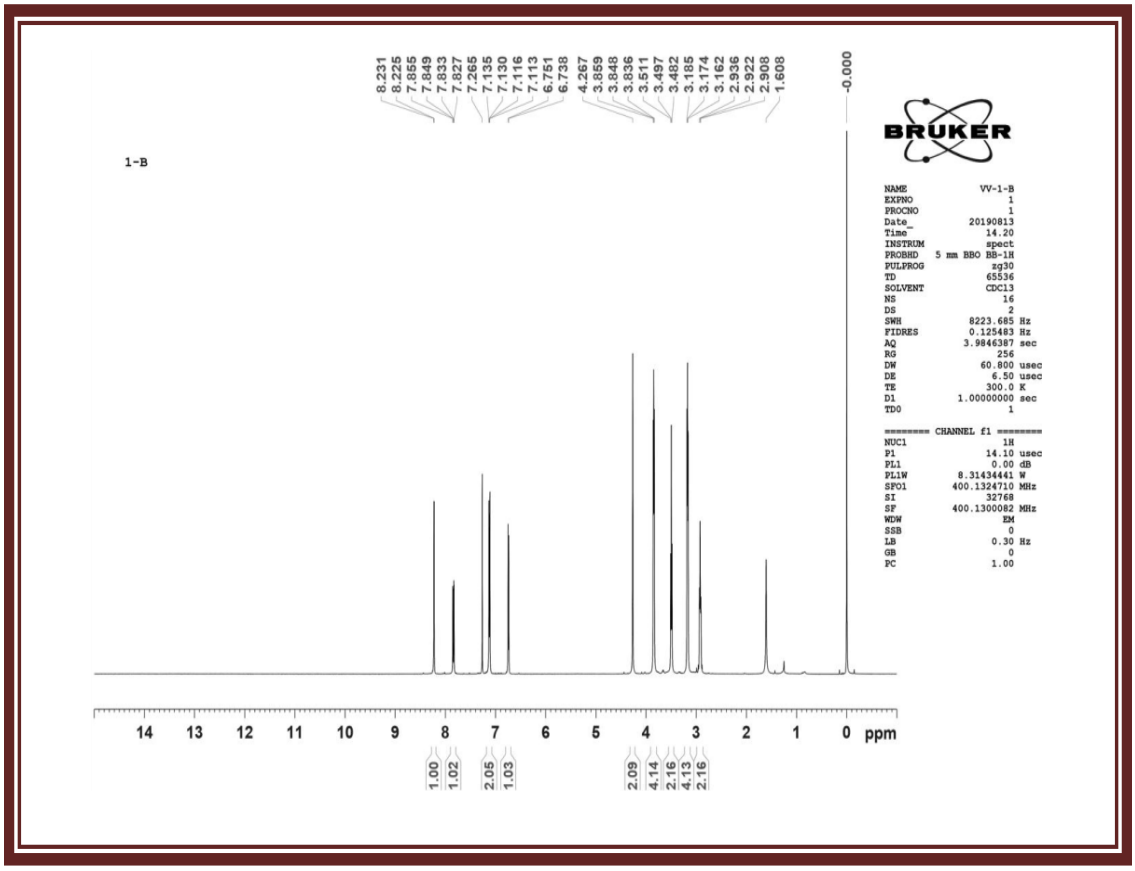


Results and Discussion:

Figure 2 IR Data of of 5–[4-(morpholin-4-yl)-3-nitrobenzene -1–Sulfonyl]- 4, 5, 6, Tetrahydrothieno [3, 2- C] Pyridine

Vibrating frequency(cm^{-1})	Functional group
3109	AromaticC–H Stretching
2978 2916 2854	AliphaticC–H Stretching
1604	C=C Stretching
1527	NO2Stretching
1342 1242	S=OStretching

^{1}H NMR Spectral study of5 - (4 –morpholin -4 –yl)- 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 – Tetrahydrothieno [3, 2 - C] Pyridine.



[1]HNMR Spectral study of5-(4–morpholin -4–yl)-3-Nitrobenzene - 1-Sulfonyl)-4,5, 6, 7–Tetrahydrothieno [3,2-C] Pyridine.

Sr.No.	SignalPosition (δ ppm)	RelativeNo. Of Protons	Multiplicity
1	8.231-8.225	1H	Doublet
2	7.855-7.827	1H	Doublet
3	7.135-7.113	2H	Doublet
4	6.751-6.738	1H	Doublet
5	4.267	2H	Singlet
6	3.859-3.836	4H	Triplet
7	3.511-3.482	2H	Triplet
8	3.185-3.162	4H	Triplet
9	2.936-2.90	2H	Triplet

[13]C NMRSpectralstudyof5- (4–morpholin-4 – yl)- 3- Nitrobenzene- 1 -Sulfonyl) - 4, 5, 6, 7 – Tetrahydrothieno [3, 2 - C] Pyridine.

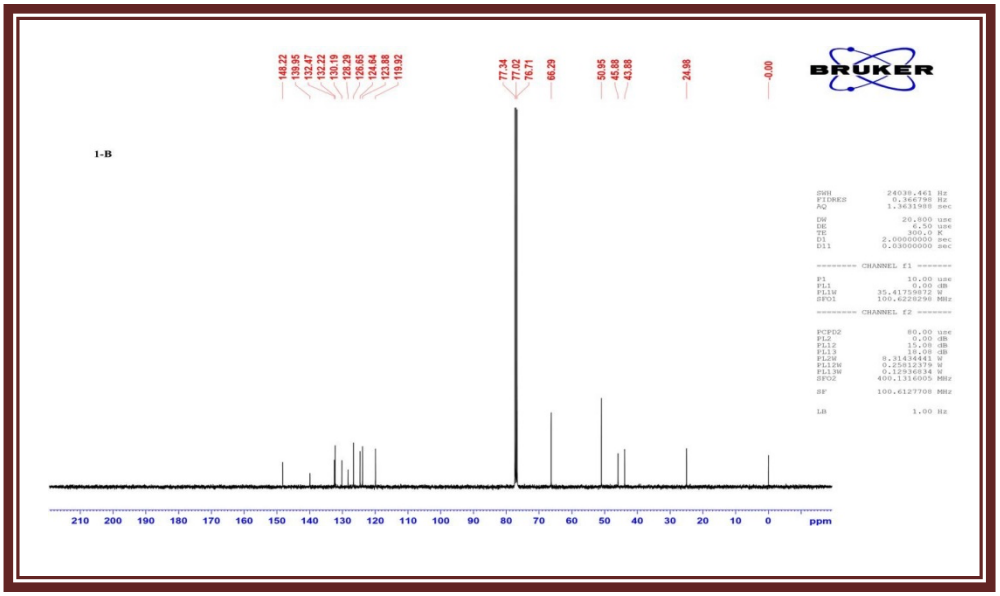


Figure: ^{13}C NMR Spectral study of5-(4–morpholin-4–yl)-3-Nitrobenzene -1-SULFONYL)-4,5,6,7–TETRAHYDROTHIENO[3,2-C] PYRIDINE.

4^{13}C NMR DATA OF5-(4–MORPHOLIN-4–YL)-3-NITROBENZENE-1-SULFONYL)-4,5,6,7–TETRAHYDROTHIENO[3,2-C]PYRIDINE.

δvalue	CarbonAssignment
24.98	14
43.88	15
45.88	11
50.95	1,4
66.29	2,3
119.92	6
123.88	9
124.64	7
126.65	16
128.29	12
130.19	13
132.22	17
132.47	8
139.95	5
148.22	10

Mass Spectral study of5 - (4 –morpholin -4 –yl)- 3- Nitrobenzene - 1 - Sulfonyl) - 4, 5, 6, 7 – Tetrahydrothieno [3, 2 - C] Pyridine.

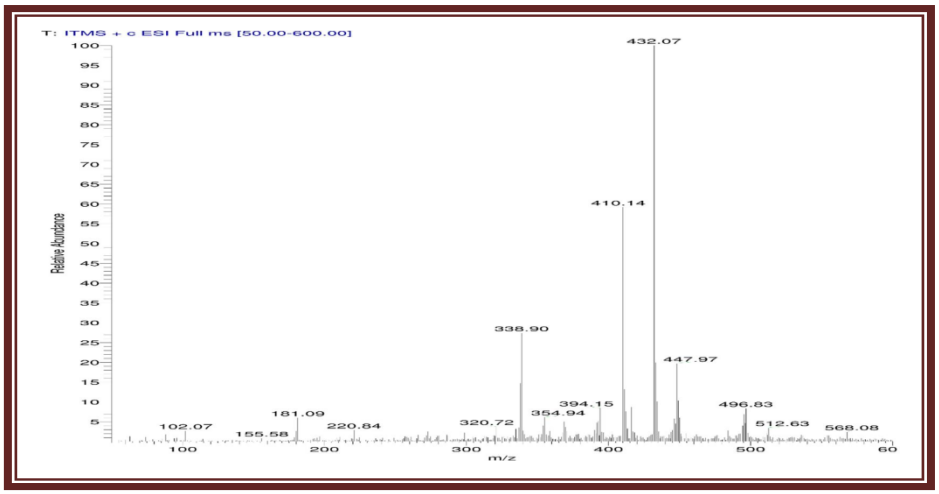


Figure: Mass Spectralstudyof5-(4–morpholin-4–yl)-3-Nitrobenzene-1 -SULFONYL)-4,5,6,7–TETRAHYDROTHIENO[3,2-C]PYRIDINE.

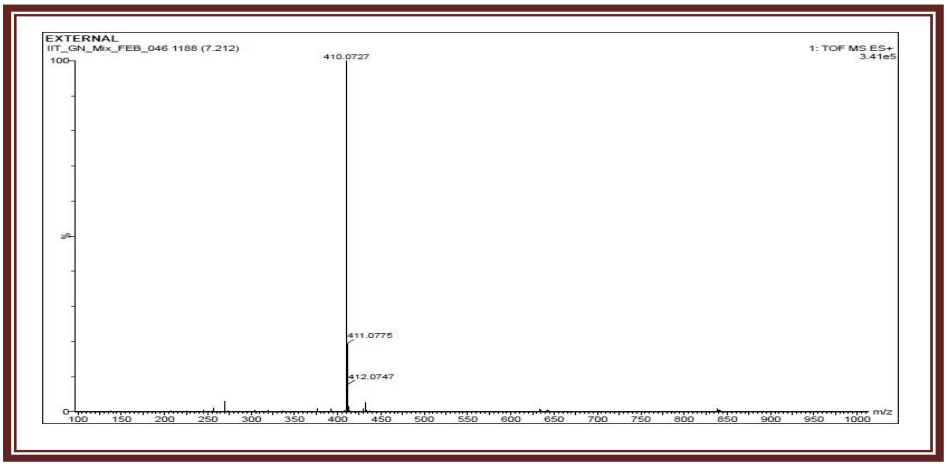


Figure: MolecularHRMSof5-(4–morpholin-4–yl)-3-Nitrobenzene-1- SULFONYL)-4,5,6,7–TETRAHYDROTHIENO[3,2-C]PYRIDINE WAS 410.14[M+1]

Antimicrobial activity

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
1B	200	150	200	250	500
Ampicillin	100	--	250	100	--
Chloramphenicol	50	50	50	50	--
Nystatin	--	--	--	--	100
Griseofulvin	--	--	--	--	100

In the series of 5-(4-morpholin-4-yl)-3-nitrobenzene-1-sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine derivatives, a compound containing a para-benzyl-substituted piperazine ring exhibited comparative inhibitory activity against E. coli when compared with the standard drug ampicillin.

However, when the piperidine and benzyl-piperazine rings were replaced with a morpholine ring, the resulting compound 1b demonstrated approximately two-fold less activity than the parent compounds.

The compound 5-(4-morpholin-4-yl)-3-nitrobenzene-1-sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine (1b) also exhibited lesser activity against P. aeruginosa compared to chloramphenicol. Specifically, compound 1b was found to be:

- Two-fold less active than compound 1c against Staphylococcus aureus, and
- Four-fold less active than chloramphenicol.

Interestingly, after introducing the six-membered morpholine ring, compound 1b showed equipotent activity against Streptococcus pyogenes when compared to ampicillin, although it remained two-fold less potent than chloramphenicol.

In contrast, replacing the six-membered morpholine ring with either a piperidine or benzyl-piperazine ring (compounds 1c and 1d, respectively) resulted in two-fold lower inhibitory activity against S. pyogenes compared to ampicillin.

When evaluating antifungal activity:

- Replacement of a five-membered unsubstituted pyrrolidine ring (compound 1a) with a six-membered unsubstituted morpholine ring (compound 1b) resulted in equipotent activity against Aspergillus niger.
- However, compound 1b remained five-fold less potent than the standard antifungal drugs nystatin and griseofulvin.

Overall, the compound 1b (5-[4-(morpholin-4-yl)-3-nitrobenzene-1-sulfonyl]-4,5,6,7-tetrahydrothieno[3,2-c] pyridine) showed lower antimicrobial activity in comparison to both its analogs and standard drugs across various tested microorganisms.

The observed data on the antimicrobial activity of the synthesized compounds and standard reference drugs are summarized in the accompanying table.

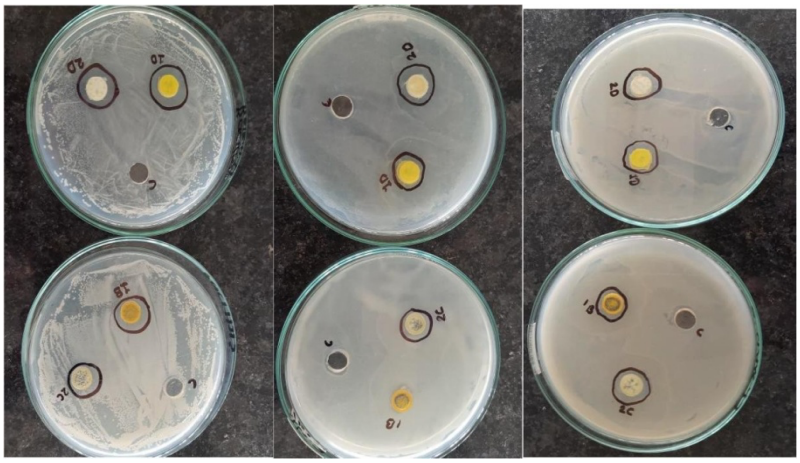


Figure: AntiMicribial acivity pictures 5-(4–morpholin-4–yl)-3- Nitrobenzene - 1- Sulfonyl) - 4, 5, 6, 7–Tetrahydrothieno [3, 2- C] Pyridine

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