Synthesis, Spectral Characterization, and In Vitro Antibacterial and Antifungal Activities of Some New Heterocycles Containing Nitrogen and Sulphur

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Abstract

The antimicrobial activity of newly synthesized linear monoazaphenothiazine derivatives; 3-benzamido-1-azaphenothiazine, 3-trifluoromethamido-1-azaphenothiazine, 3-trichloromethanamido-1-azaphenothiazine and 3-(4-nitrobenzamido)-1- azaphenothiazine have been evaluated against bacteria and fungal strains. Results demonstrated that majority of the linear monoazaphenothiazine derivatives were very active against the tested bacteria. The association between antibacterial action and the chemical structure of phenothiazines was explored. Thiazine moieties contained in compounds show variety of therapeutic properties such as antihypertensive, antitumor, antibiotic, antibacterial, anticoagulant, antifungal, anticancer, and antiviral. This review is largely centered on thiazines and their derivatives with potential antibacterial activity that are at this moment in progress.

Keywords: *phenothiazine; monoazaphenothiazine, microorganisms, antimicrobial, structure – activity relationship.*

1. INTRODUCTION

The relevance of heterocyclic compounds with biological activity has grown in recent years. (1) The pharmaceutical world has long been interested in heterocyclic compounds for their medicinal usefulness, with a focus on those with five or six members. (2) Heterocyclic compounds with several functional groups, such as those with nitrogen, sulphur, or oxygen as a heteroatom, are crucial to the development of new therapeutics. According to the data, around 68% of currently available medications or those in the final phases of clinical testing are heterocycles. Diuretic, antiprotozoal, cardiotonic, fungicidal, sedative, anesthetic, antimalarial, central nervous system (CNS) depressant, hypoglycemic, inflammatory, and antimicrobial activities are just some of the biological properties of 1,3,4-thiadiazole derivatives that have attracted widespread interest and investigation in recent years1-5. Therefore, the goal of this study was to synthesize novel substituted 1,3,4 thiadiazole derivative heterocycles for the purpose of developing less hazardous medicines for anti-tubercular and anti-diabetic applications. New insights into the synthesis and pharmacological effect of fused heterocycles like pyrazolines and similar heterocyclic compounds have been added to the present body of literature. Because of the ferrocenyl moiety's stability and lack of toxicity, these medications may be used in conjunction with other treatments, which is of great importance (Biot et al., 2000). Adding one or more ferrocene units to a heterocyclic molecule is a popular strategy for doing so. This allows for the creation of new molecules with interesting properties.

2. LITERATURE REVIEW

Monika Shirke et.al (2021) It has been observed that 4-thiazolidinones based on biphenyls have a broad variety of actions, which has led to their production. Using four separate processes, eight molecules (VIIa-VIIh) were synthesized in great detail. Elemental (CHN) and spectroscopic (IR, 1H NMR) studies were used to describe these 4-thiazolidinone derivatives. One Gram-negative strain (Escherichia coli), two Gram-positive strains (Bacillus subtilis, and Staphylococcus aureus), and two fungus strains were used to test the compounds' in vitro

antibacterial properties (Candida albicans and Aspergillus niger). Antimicrobial investigations indicated that compound VIIg had weak antifungal activity, compound VIIc had moderate antibacterial activity, and compound VIIe had strong antibacterial activity against a bacterial strain. Antimicrobial activity was shown to be enhanced in compounds having electron-withdrawing groups (-NO2, -CI, -Br) on the aromatic ring.

Mahmoud S. Tolba et.al (2021) Due to their biological significance in the fight against microbes, heterocyclic compounds play a significant part in our daily lives. From the difunctionalized molecule 5-amino-4-phenyl-2-(p-tolylamino) thieno [2,3- d] pyrimidine-6-carbonitrile, a series of new hybrid compounds of thienopyrimidine with triazine and pyrimidine scaffolds were synthesised (1). Further, the chloro-triazine compound 2 was obtained through diazotization of compound 1 with sodium nitrite in an acidic medium, and compounds 3a-5c were obtained via nucleophilic replacement of the chlorine atom with various nucleophiles. Additionally, compound 1 was reacted with carbon disulfide to produce dithione derivative 6, which was subsequently alkylated with ethyl chloroacetate to yield compound 7. Compound 8 was obtained by reacting compound 1 with phenyl isothiocyanate, and compound 9 was obtained by acylating the amino group in compound 1 with acetic anhydride. Element and spectral analysis methods were used to characterize all produced substances (IR, 1H NMR, 13C NMR, Mass spectroscopy). Additional testing of the synthesized compounds for antibacterial activity against a variety of bacterial and fungal strains revealed excellent to moderate activity with virtually all of the strains tested.

Nagaraju Kerru et.al (2020) In the field of medicinal chemistry, heterocycles based on nitrogen are unique in their ability to provide useful analogs for the development of therapeutic medicines. Over seventy-five percent of all FDA-approved medications include nitrogen heterocyclic moieties. New medications based on nitrogen are expected to make up a substantially larger percentage of the market in the future decade. There have been a lot of attempts to build novel heterocycles based on nitrogen. A rising body of research has identified a wide variety of new N-heterocyclic moieties that show great promise for use in medicinal chemistry due to their potent physiological effects. Here, we provide a comprehensive overview of the most up-to-date research on new nitrogen-containing heterocycles and the varied biological activities they exhibit during the previous year (2019 to early 2020). In this article, we will discuss the current trends in the utilization of nitrogen-based moieties in drug design and the progress made in creating effective and competent candidates against diverse illnesses.

Thierry Y Fonkui et.al (2018) Food deterioration and treatment difficulties due to microbial resistance to existing medications have contributed to a rise in worldwide death rates. Among the many classes of chemical compounds, Schiff bases stand out for their adaptability and usefulness in the pharmaceutical industry due to their significant pharmacological characteristics. To create them, a primary amine and a carbonyl are typically condensed. They show promising antibacterial efficacy against a broad variety of microorganisms, including fungus, bacteria, parasites, and viruses. Schiff base ligands' chelating behavior enhances their antibacterial action, hence complexing them with metals is a common way to boost this property. Schiff bases and the metal complexes they form are known to be easily synthesized. That's why it's crucial to classify and assemble them according to their biological relevance. Some chosen heterocyclic Schiff bases and their metal complexes are described in this study for their antibacterial, antifungal, antiparasitic, and antiviral activities.

S. R. DESHPANDE ety.al (2017) Through the use of molecular hybridization, new 2-(benzimidazol-2-ylthiomethyl)-5-aryl-1,3,4-oxadizoles and 1-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl)pyridazine/phthalazinediones were developed, synthesized, and characterized to shed light on the biological significance of heterocyclic cores. Using the serial dilution method, the compounds were examined for in vitro antimicrobial activity and shown to have low antitubercular activity, high antibacterial activity, and superior antifungal activity than certain reference medications against some of the tested species. Therefore, several of the substances named in the title exhibited antibacterial activity.

3. EXPERIMENTAL DATA

There was no further purification of any of the chemicals used in this investigation since they were all of Analytical Grade (AR) purity. Every one of the amidation reactions was performed in a nitrogen environment. The 2,3,5-trichloropyridine, 2-aminothiophenol, trichloroacetamide, trifluoroacetamide, benzamide, nitrobenzamide, potassium trioxocarbonate(IV), terbutylhydroxide, nickel(II)chloride, ethyl acetate, and triphenylphosphine ligand were all purchased from Zayo-Sigma Chemicals, Germany and used directly from their Sonication of distilled water for 30 seconds under a vacuum degassed the water. Merck's Silica Gel was used for the column chromatography purification of the produced 3-amidoderivatives (60-230 Mesh). The

created analogues' melting points were measured using a Fisher-Johns apparatus, and these readings were made without any corrections.

The 4000-400 cm-1 area of the FT-IR spectra of the produced compounds was measured using a SPECTRUM RX I spectrophotometer (Perkin Elmer). The imidazole 1 H NMR spectra were acquired at room temperature on a Bruker 400 NMR spectrometer operating at 400 MHz. Imidazole 13C NMR spectra were acquired at room temperature using a Bruker 400 NMR spectrometer with proton decoupling.

3. RESULT

Paper disc diffusion assays were performed using Muellere Hinton agar and Sabouraud dextrose agar culture media to test all of the produced analogues for in vitro antibacterial activity. Anti-microbial activities of these analogues were determined by measuring their zones of inhibition against a collection of gram-positive, gram-negative, and fungal organisms, including S. aureus, B. subtitis, E. coli, P. aeruginosa, A. niger, and C. albican, among others. Paper discs impregnated with various concentrations of the produced analogues and the reference medication in ethanol were used to seed the culture medium, which was then incubated at 37 O C for 48 hours. In this case, Ciprofloxacin and ketoconazole were used as examples of effective antibacterial and antifungal drugs. The Petri plate had two distinct environments: the test group and the solvent control.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a produced analogue observed to impede the observable growth of a certain microbe following incubation in a suitable culture medium for a predetermined amount of time. The minimal inhibitory concentration (MIC) of the produced analogues was evaluated using the solid dilution technique with a series of petri plates containing an appropriate growth medium. A very effective counterpart significantly reduces the microbe's proliferation even at low concentrations. The microorganisms can be killed by low concentrations of an antimicrobial agent, whereas large concentrations are required for the less effective agents. Here, the anti-microbial activity of the produced analogues (compounds 1-4) in ethanol at varying concentrations against various micro-organisms were determined during a 48-hour incubation at 37 OC.

Table 1: Zone of inhibition of synthesized compound against different micro-organism (mm).

Compound(s)	Gram Positive Bacteria		Gram negative bacteria		Fungi	
	S. aureus	*B.subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albican*
1	**15**	**24**	**20**	**10**	-	-
2	**24**	**22**	**27**	**05**	-	**24**
3	**26**	**16**	**32**	**16**	**18**	**21**
4	**28**	**21**	**31**	**36**	**24**	**19**
CPFX	**30**	**27**	**32**	**34**	-	-
KTNZ	-	-	-	-	**22**	**24**
ETHANOL	-	-	-	-	-	-

Table 2: Results of the Minimum Inhibitory Test (Summary of the antibacterial and antifungal effects of synthesized compounds (concentrations in µg/ml))

Compounds	*S. Aureus*	*B subtitis*	*E. coli*	*P. Aeruginosa*	*A niger*	*C. albican*
1	20	05	16	32	-	-
2	08	08	05	16	03	03
3	05	14	03	04	06	04
4	04	16	02	02	02	08
Ciprofloxacin	03	04	03	-	-	-
Ketoconazole	-	-	-	-	03	05

Among the gram-negative bacteria, compounds 3 and 4 showed the greatest action against P. aeruginosa, with respective MIC values of 4 ug/mL and 2 mg/mL and zones of inhibition of 34 mm and 36 mm. Compounds 3 and 4 likewise shown remarkable activity against E. coli, with MIC values of 3 ug/mL and 2 mg/mL and zones

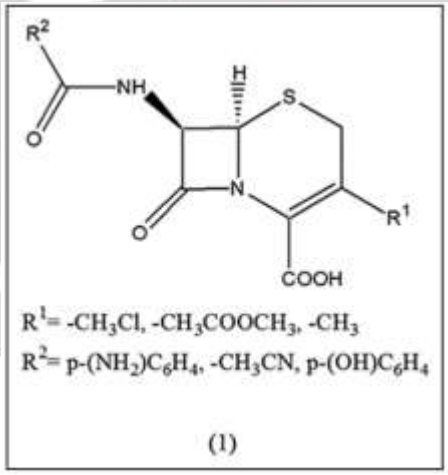
of inhibition of 31 mm and 32 mm, respectively. Compounds 2, 3, and 4 showed the greatest efficacy against S. aureus, a gram-negative bacterium, with respective MIC values of 8 ug/mL, 5 ug/mL, and 4ug/mL and zones of inhibition of 24 mm, 26 mm, and 28 mm. Compound 1, on the other hand, was effective against the bacterium strain, however only at a very high dosage of 20 ug/mL and a very small zone of inhibition of 15 mm. Compounds 2 and 3 had the strongest activity against A. niger of the fungi tested, with MIC values of 3 ug/mL and 6 ug/mL, respectively, while Compound 4 had the lowest MIC value of 2 ug/mL. Compound 1 was ineffective against the tested fungus. Compounds 3 and 4 showed the greatest activity against C. albican at MIC values of 4 g/mL and 8 g/mL, respectively. Compound 4's MIC against the fungal strain was 4.2 ug/mL, and its zone of inhibition was 21 mm. Neither compound 1 nor compound 2 had any effect on the C. albican. Tables 1 and 2 describe the in vitro antibacterial outcomes of all of the produced compounds.

4. IR SPECTROSCOPY

The term "infrared" (IR) radiation describes the portion of the electromagnetic spectrum that is between the visible and microwave ranges. The smaller range between 4000 and 400 cm-1 is of more practical utility to organic chemists. Molecular vibrations in the stretching and bending modes of a bond cause energy shifts, which in turn cause absorption bands in the spectrum. Because a single change in vibrational energy is accompanied by several changes in rotational energy, the vibrational spectra appear as bands rather than a line, despite the quantization of the absorption. Infrared spectra typically display band locations in terms of wavenumber () or wavelength (). It is possible to get a very complicated infrared spectrum from even a very basic organic molecule. The organic chemist used this intricacy to his advantage by comparing the unknown compound's spectrum to that of a recognized one. Correlation between individual peaks provides strong indication of similarity or identity. Except for enantiomers, it is quite improbable that two substances would have identical infrared spectra. In this investigation, we use IR spectra in conjunction with other spectral data to infer the molecular structure.

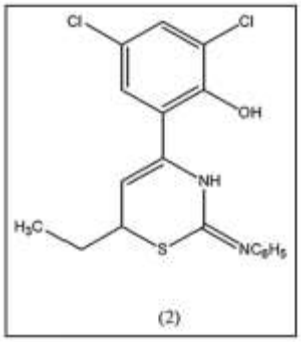
5. ANTIMICROBIAL ACTIVITIES OF NITROGEN AND SULFUR CONTAINING HETEROCYCLES

Antimicrobial efficacy against many bacterial strains was shown by heterocycles based on 1,3-thiazines, as suggested by Damanjit (2013).



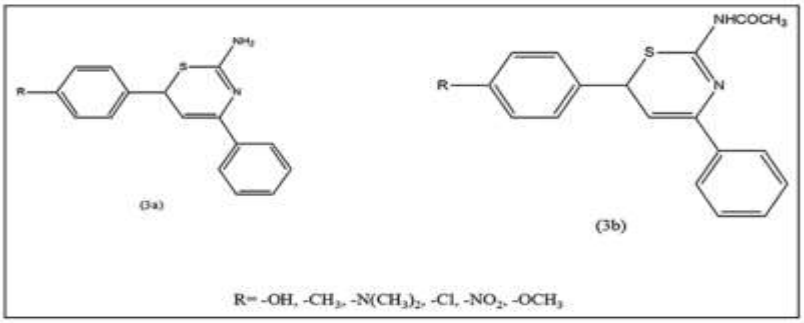
(1)

Antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa was shown for 1,3-thiazines produced from chalcones.

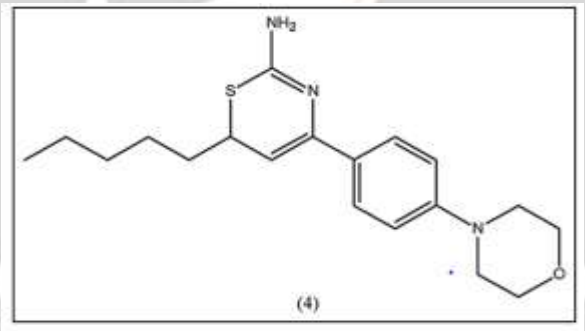


(2)

Antimicrobial activity of 1,3-thiazine compounds and their acylated metabolites was discovered by Bhangale and Wadekar in 2011.

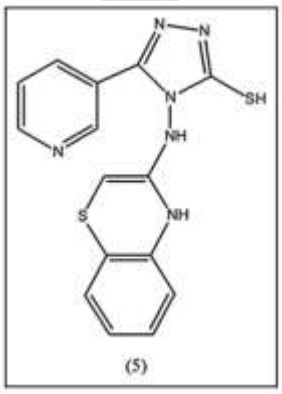


Morpholine thiazine compounds were discovered to have potent antifungal and antibacterial action against Rhizopus and Vibrio cholerae, respectively, in a study conducted by Thanusu and Gopalakrishnan in 2010.



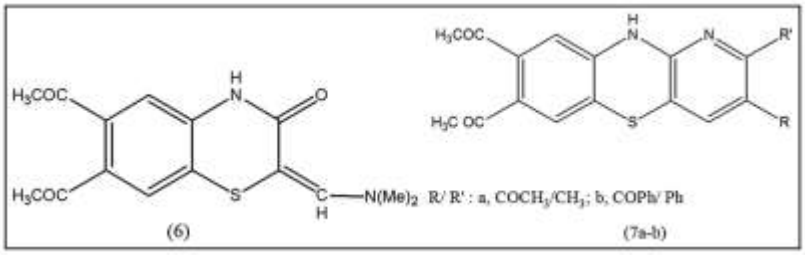
(4)

shown good antibacterial activity when compound 5 was tested against two microorganisms, E. coli and S. aureus.



(5)

Compounds 6, 7a, and 7b were tested by Abbas and Farghaly (2010) and shown to be effective against S. aureus and (E. coli), as well as the pathogenic fungus Aspergillus flavus and Candida albicans.



6. CONCLUSION

The synthetic derivatives have variable degrees of activity against the various bacterial and fungal strains that were cultivated. To my surprise, compounds 3 and 4 were more effective than the reference medication in killing E. coli and P.aeruginosa. Some of the synthetic equivalents, however, lacked the efficacy of gold-standard antibiotics (Ciprofloxacin). It is important to highlight that Ketoconazole, the gold standard antifungal medicine, was less effective against A. niger than compounds 3 and 4. Against C.albican, Compound 2 is just as effective as the control. The most active compounds were identified, and they are the ones that should be suggested for more preclinical screening, as they may be effective in the fight against bacterial and fungal diseases. According to the research, thiazines are the most important class of heterocyclic chemicals, and their effects are particularly problematic in pathogen-based diseases. The thiazine moiety has attracted a great deal of interest from biochemists and medicinal chemists, and this review of thiazine-based heterocycles has shown that it has the potential to be an essential molecule in the creation of new biologically active pharmaceutical molecules.

7. REFERENCE

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