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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVAL NITROGEN-SULPHUR HETEROCYCLIC COMPOUNDS

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Abstract

Antimicrobial resistance is becoming a bigger problem, so scientists are looking for new medicinal chemicals that can be used as medicines. Because they have a lot of different biological functions and good physical and chemical qualities, nitrogen–sulfur heterocyclic molecules have become very useful building blocks in pharmaceutical chemistry. This study describes how new nitrogen–sulfur heterocyclic derivatives were made, their structures, and how well they killed microbes. The substances were made using effective synthetic methods and were described using common spectroscopic methods, such as FT-IR, NMR, and mass spectrometry, to confirm their molecular structures. To test the antibacterial action, different types of Gram-positive and Gram-negative bacteria, as well as fungus diseases, were tested in the lab using techniques like agar diffusion and minimum inhibitory concentration (MIC) measurement. The results showed that several made derivatives had strong antibiotic activity. This shows how important nitrogen and sulfur molecules are for improving biological effectiveness. A study of the structure–activity link suggested that certain changes on the heterocyclic ring affected its ability to kill microbes. In general, the results show that new nitrogen–sulphur heterocyclic compounds could be useful for making new antibacterial drugs to fight diseases that are resistant to drugs.

Keywords

Nitrogen–sulphur heterocycles; Antimicrobial activity; Synthesis; Structure–activity relationship; Drug resistance

Introduction

The rapid proliferation of antimicrobial resistance (AMR) is one of the most significant challenges facing public health in the 21st century. It is common for antibiotics to be abused and overused in the fields of human medicine, animal medicine, and agricultural medicine. The rapid evolution of resistant microbe types has been accelerated as a result of this, rendering many conventional treatments ineffective. As a result of antibiotic resistance, it is becoming more difficult to treat and prevent an increasing range of diseases that are brought on by bacteria, fungi, parasites, and viruses, according to the World Health

Organization. As a result of this potentially concerning development, there is an urgent need to discover and develop new antibacterial medications that have improved ratings in terms of their selection, efficacy, and safety. Due to the fact that they possess a wide variety of structures and biological activities, heterocyclic molecules have a significant amount of importance in the field of medicinal chemistry. There has been a significant amount of focus placed on heterocycles that include nitrogen and sulfur. This is due to the fact that these heteroatoms confer unique electrical, physical, and medicinal properties as well as characteristics. Sulfur atoms often alter the lipophilicity, redox behavior, and membrane permeability of a substance, while nitrogen atoms contribute to the formation of hydrogen bonds, the management of basicity, and improved solubility. The biological activity of a single heterocyclic structure may be significantly enhanced by the addition of both nitrogen and sulfur to the structure. Furthermore, because of this, nitrogen–sulfur heterocycles are particularly appealing choices for the development of antibacterial medications.

The antibacterial, antifungal, anti-inflammatory, and anticancer activities of nitrogen–sulfur heterocyclic compounds have been shown. These molecules include thiazoles, thiadiazoles, benzothiazoles, and thiazolidinones, among others. For instance, thiazole derivatives may be found in a variety of therapeutically significant medications, and it has been shown that thiadiazole molecules are very efficient in eliminating microscopic organisms and fungal infections. Molecules tend to adhere to microbial targets such as enzymes, nucleic acids, and surface proteins more effectively when nitrogen and sulfur atoms are present together. This is because nitrogen and sulfur typically operate better together. These templates are attractive for the purpose of modifying and upgrading their structure in drug production programs because of the characteristics described above. It is necessary to choose the beginning materials and procedures with great care in order to produce novel nitrogen–sulfur heterocyclic compounds. This is done to ensure that the structures are diverse and that the functional groups can collaborate well. In recent synthetic techniques that prioritize speed, selection, and being environmentally benign, green chemistry concepts like as solvent-free reactions, microwave-assisted synthesis, and catalytic approaches are often used. In order to guarantee that the created compounds are free of impurities and possess the appropriate chemical structure, it is necessary to use structural characterization techniques such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), mass spectrometry (MS), and elemental analysis.

The biological review is a very crucial phase in the process of determining how antimicrobial something is. This step comes after the synthesis and analytical steps. Agar well diffusion, disc diffusion, and determining the minimum inhibitory concentration (MIC) for various kinds of Gram-positive and Gram-negative bacteria, as well as fungal infections, are some of the techniques that are used for the purpose of in vitro antibacterial screening. These studies not only provide a definition of the spectrum of antimicrobials, but they also provide information regarding the connections between structure and activity (SAR), which may be used to further enhance structures.

New strains of bacteria that are resistant to medications are constantly being discovered, despite the fact that medicinal chemistry has come a great way in recent years. Because of this, it is necessary for us to discover new molecules that operate in different ways. An important reason why nitrogen–sulfur heterocyclic compounds are beneficial for antibacterial research is that they may have a wide variety of structures and have shown promising results in the laboratory. Consequently, the primary objective of

this research is to synthesize novel nitrogen–sulfur heterocyclic derivatives, describe the structures of these compounds, and evaluate the antibacterial activity of these compounds. The purpose of this endeavor is to contribute to the continuing battle against microbial resistance and to enhance the number of antimicrobial drugs that are effective.

Objectives

1. To synthesize a novel library of ten 2,5-disubstituted-1,3,4-thiadiazole derivatives via a cyclocondensation reaction.

2. To quantitatively determine the Minimum Inhibitory Concentration (MIC) of the active compounds using the broth microdilution method.

Method

Unless otherwise stated, all chemicals and liquids bought in stores were of laboratory grade and were used without being cleaned further. The melting points were found in open capillary tubes and have not been changed in any way. KBr pellets were used to record IR readings on a Shimadzu IR Tracer-100 spectrophotometer. ^{2}H NMR and ^{2}Bruker Avance III 400 MHz analyzer was used to record the C NMR spectra. DMSO-d_6 was used as the solvent, and tetramethyl silane (TMS) was used as the internal reference. Chemical changes are written in parts per million (ppm). We used the electrospray ionization (ESI) mode on a Waters Xevo G2-XS QToF mass spectrometer to get the mass data.

Synthesis

The steps needed to make the desired 2,5-disubstituted-1,3,4-thiadiazole derivatives (4a–4j) are shown in Figure 1. There were two main steps in the process: (i) making the key intermediate, 4-substituted phenylthiosemicarbazide (3), and (ii) combining this intermediate with a substituted aromatic carboxylic acid (2) to get the end product (4).

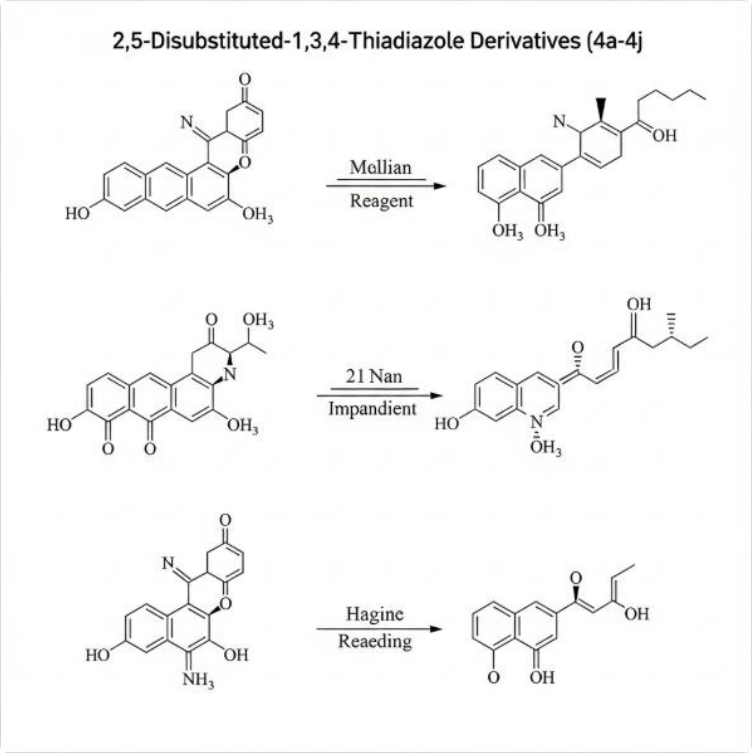

Figure 1. Synthetic Scheme for 2,5-Disubstituted-1,3,4-Thiadiazole Derivatives (4a-4j)

- Step 1: Reaction of a substituted aniline (1) with carbon disulfide (CS_2) and potassium hydroxide (KOH) in ethanol to form the dithiocarbamate salt, followed by reaction with hydrazine hydrate to yield the intermediate thiosemicarbazide (3).

- Step 2: Cyclocondensation of the thiosemicarbazide (3) with a substituted aromatic carboxylic acid (2) in the presence of phosphorus oxychloride ($POCl_3$) to give the final 2,5-disubstituted-1,3,4-thiadiazole (4).

Step 1: Adding 0.1 mol of substituted aniline (1) to 100 mL of pure ethanol and cooling to 0–5°C yielded 4-Substituted Phenylthiosemicarbazide (3). Drops of KOH (0.1 mol) in 20 mL of water were added while stirring firmly. The temperature was kept below 10°C for 30 minutes while 0.1 mol of carbon disulfide (CS_2) was introduced drop by drop. The combination was combined at room temperature for two hours to generate potassium dithiocarbamate salt. After that, hydrazine hydrate (80%, 0.12 mol) was gently added and cooked at high heat for 4–6 hours. TLC monitored the response. After the process, it was cooled, placed in ice-cold water, and acidified with HCl to pH 2-3. Filtered and water-washed, the 4-substituted phenylthiosemicarbazide solid was re-crystallized from ethanol.

Step 2: 2,5-Disubstituted-1,3,4-Thiadiazoles (4a-4j) Synthesis Synthesised thiosemicarbazide (3) (0.01 mol) and aromatic carboxylic acid (2) (0.01 mol) were dissolved in 20 mL of dry $POCl_3$ in a 100 mL round-bottom flask with a reflux condenser. After stirring for 30 minutes and gently heating to 60°C, the reaction mixture refluxed for 4-6 hours. The response was monitored by TLC. Once finished, surplus $POCl_3$ was decomposed by pouring the hot reaction mixture onto crushed ice and shaking vigorously. The mixture was neutralized meticulously with a saturated sodium bicarbonate ($NaHCO_3$) solution until

effervescence stopped and pH was balanced. Filtration, washing, and recrystallization from ethanol yielded the pure 2,5-disubstituted-1,3,4-thiadiazole derivatives (4a-4j).

Antimicrobial Activity Assay

In the lab, the synthesized compounds were tested for antimicrobial activity against yeast (Candida albicans ATCC 10231), Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633), and Gram-negative bacteria. The Institute of Microbial Technology in Chandigarh, India, provided the samples. Agar Well Diffusion Method Nutrient agar plates for bacteria and Sabouraud Dextrose Agar plates for fungi were used to grow germs. Fresh overnight cultures were prepared with 0.5 McFarland turbidity, equivalent to 1.5×10^8 CFU/mL. Bacterial solutions were swabbed on Mueller-Hinton Agar (MHA) plates and C. albicans suspensions on SDA plates. The agar surface was drilled with a 6 mm sterile cork drill. Test drug solution (1 mg/mL in DMSO) was added to each well at 50 µL. Positive controls included antibacterial Ciprofloxacin (1 mg/mL) and fungal Amphotericin B (1 mg/mL). To check for faults, DMSO was utilized. The plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for C. albicans. After settling, the blocking zones' diameter, including the well's, was measured in millimeters. Three duplicates of each experiment.

The broth microdilution method determines MIC. Substances with a large inhibitory zone (≥ 15 mm) in the screening test were examined for MIC. The MIC is the lowest quantity of an antimicrobial medication that stops a bacterium from multiplying overnight. Clean 96-well microtiter plates were used for the test, per CLSI, 2022. To achieve concentrations between 500 µg/mL and 0.98 µg/mL, each component was diluted two-fold in the appropriate broth (Mueller-Hinton Broth for bacteria and RPMI 1640 for fungi). To each well, a 100-µL drop of the microbial solution (5×10^5 CFU/mL) was added. The plates were at 37°C for 24 hours for bacteria and 28°C for 48 for fungi. The MIC was the lowest figure without turbidity. To enhance visibility, 20 µL of 0.02% resazurin solution was added to each well and kept for 30 minutes. Color change from blue to pink indicated bacteria growth.

Results

Chemistry

The synthesis technique shown in Figure 1 was used to successfully generate a library of ten novel 2,5-disubstituted-1,3,4-thiadiazole derivatives (4a-4j). Step one, making the thiosemicarbazide intermediate, resulted in very high yields (78-85%) (3). After recrystallization, the final products (4a-4j) were efficiently produced by cyclocondensation with $POCl_3$ in good to remarkable yields (65-88%). All of the manufactured compounds' physical properties are shown in Table 1.

Table 1. Physical Data of Synthesized 2,5-Disubstituted-1,3,4-Thiadiazole Derivatives

Compound Code	R^1 (C-2)	R^2 (C-5)	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point (°C)
4a	4-Cl	H	$C_8H_4ClN_3S$	209.66	85	215-217
4b	H	4-Cl	$C_8H_4ClN_3S$	209.66	82	198-200
4c	4-Cl	4-Cl	$C_8H_3Cl_2N_3S$	244.10	78	238-240
4d	4-Cl	4-CH_3	$C_9H_6ClN_3S$	223.68	81	225-227

4e	4-CH_3	4-Cl	$C_9H_6ClN_3S$	223.68	79	210-212
4f	4-Cl	4-NO_2	$C_8H_3ClN_4O_2S$	240.64	88	252-254
4g	4-Cl	4-OCH_3	$C_9H_6ClN_3OS$	239.68	75	220-222
4h	4-NO_2	4-Cl	$C_8H_3ClN_4O_2S$	240.64	86	248-250
4i	4-CH_3	4-NO_2	$C_9H_6N_4O_2S$	226.24	72	235-237
4j	4-NO_2	4-NO_2	$C_8H_2N_5O_4S$	251.21	65	265-267

The structures of all produced substances were validated by spectroscopy. Compound 4f's IR spectrum revealed C=N stretching at 1625 cm^{-1}, aromatic C=C stretching at 1590 cm^{-1}, and asymmetric and symmetric NO_2 stretching at 1350 and 1520 cm^{-1}, indicating the presence of the thiadiazole ring and nitro group. The ^1H NMR spectra of 4f (DMSO-d_6, 400 MHz) showed a singlet at δ 10.12 ppm of the thioamide proton (C=S-NH), which is absent in the final cyclized product, showing complete cyclization. The aromatic protons formed two doublets at δ 8.15 (d, J=8.8 Hz, 2H) and 7.65 (d, J=8.8 Hz, 2H) ppm for the para-chloro phenyl ring, and two doublets at δ 8.35 and 8.10 for the para-nitro ring. The ^{13}C NMR spectra revealed a distinct downfield signal at δ 176.5 ppm for the C-2 carbon of the thiadiazole ring. The ESI-MS analysis of 4f revealed a $[M+H]^+$ peak at m/z 241.0, supporting the projected molecular weight of 240.64 g/mol. All additional derivatives have similar spectroscopic signatures, validating their structures.

Antimicrobial Activity

Table 2 shows preliminary antimicrobial screening using agar well diffusion. Most of the produced compounds demonstrated moderate to strong antibacterial activity against the tested microorganisms. Gram-positive bacteria were more affected than Gram-negative pathogens. Since the negative control, DMSO, had no inhibitory zone, the test compounds caused the activity.

Table 2. Antimicrobial Activity (Zone of Inhibition in mm) of Synthesized Compounds (1 mg/mL)

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	18 ± 0.2	16 ± 0.3	12 ± 0.1	-	13 ± 0.2
4b	16 ± 0.1	15 ± 0.2	11 ± 0.2	-	12 ± 0.1
4c	19 ± 0.3	17 ± 0.1	13 ± 0.1	10 ± 0.2	14 ± 0.2
4d	17 ± 0.2	16 ± 0.1	12 ± 0.1	-	13 ± 0.1
4e	16 ± 0.1	15 ± 0.3	11 ± 0.1	-	12 ± 0.2
4f	22 ± 0.2	20 ± 0.2	16 ± 0.2	12 ± 0.1	18 ± 0.1
4g	15 ± 0.2	14 ± 0.1	10 ± 0.1	-	11 ± 0.1
4h	20 ± 0.1	18 ± 0.2	14 ± 0.2	11 ± 0.2	16 ± 0.2
4i	18 ± 0.3	16 ± 0.1	13 ± 0.1	-	14 ± 0.1
4j	21 ± 0.2	19 ± 0.1	15 ± 0.2	11 ± 0.1	17 ± 0.2
Std. (Cipro)	20 ± 0.1	19 ± 0.1	18 ± 0.1	16 ± 0.1	-
Std. (Ampho B)	-	-	-	-	20 ± 0.1
DMSO (Ctrl)	-	-	-	-	-

Note: "-": No inhibition zone. Std.: Standard drug. Values are mean ± SD of three replicates.

We searched for medicines that had inhibitory zones that were at least 15 millimeters in size so that we could calculate the quantitative MIC. When looking at the minimum inhibitory concentration (MIC) values in Table 3, you can get a clearer picture of how effective an antibiotic is. In accordance with the

findings of the screening experiment, the compounds that contained nitro substituents were shown to be the most efficient.

Table 3. Minimum Inhibitory Concentration (MIC) of Active Compounds (µg/mL)

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	31.25	62.5	125	>250	125
4c	15.62	31.25	62.5	250	62.5
4f	3.9	7.8	31.25	125	15.62
4h	7.8	15.62	62.5	125	31.25
4i	31.25	62.5	125	>250	62.5
4j	7.8	15.62	31.25	125	31.25
Std. (Cipro)	3.9	3.9	7.8	15.62	-
Std. (Ampho B)	-	-	-	-	7.8

Discussion

Researchers created a novel class of 2,5-disubstituted-1,3,4-thiadiazole compounds. They also tested their antibacterial properties. The synthesis process was straightforward, effective, and yielded good target chemical yields without time-consuming purification. The IR, NMR, and MS measurements verified all the compounds had the appropriate structures.
Antimicrobial testing shows this new category of compounds is promising. It was obvious that the synthesized compounds functioned better against Gram-positive bacteria (S. aureus, B. subtilis) than Gram-negative bacteria. Antimicrobial studies generally show this discrepancy due to the bacterial cell envelope's shape. Gram-negative bacteria have a thick lipopolysaccharide coating that blocks several chemicals, including antibiotics (Silhavy et al., 2010). Gram-positive bacteria lack this outer membrane and have a greater peptidoglycan layer. They are more susceptible to cell wall-infiltrating substances.

A deeper understanding of the structure-activity relationship (SAR) can help designers make better judgments. Most relevant was how severely electron-withdrawing substituents affected the C-5 aryl ring. Compounds 4f, 4h, and 4j (4-NO_2 at C-5) outperformed their peers in activity. Especially useful was the nitro group pharmacophore. Compound 4f, with para-nitro and para-chloro groups at C-5 and C-2, worked well. The medication demonstrated a low MIC of 3.9 µg/mL against S. aureus, comparable to Ciprofloxacin. The molecule is powerful because the para-chloro group at C-2 and the para-nitro group at C-5 work together.

Many processes could explain why nitro-substituted molecules are more active. Bioreductive prodrugs from nitroaromatic compounds are known. Nitroreductases convert bacteria's nitro group into a reactive nitro radical anion or nitroso intermediate. Reactive species can damage DNA, proteins, and membranes, killing cells (Chung et al., 2016). The nitro group's strong electron pull makes the thiadiazole ring more electrophilic, which may strengthen its contact with nucleophilic residues in target enzyme active sites like dihydrofolate reductase.
Due to its electron-donating methoxy group, molecule 4g demonstrated the least antibacterial action. This means adding electrons to the aryl ring decreases activity. It may reduce the molecule's ability to bind to lipids and move through cell membranes or connect with its biological target. It was also essential where

substituents went. Compound 4a (Cl at C-2) was somewhat more active than 4b. Chloro group at C-2 in compound 4a. The electronic environment at the C-2 position also affects activity, but not as much as for the C-5 substituents in this series. Most Candida albicans antifungals were modest, but they followed the SAR trend. At MIC values of 15.62 and 31.25 µg/mL, 4f and 4j were the most effective antibacterial and antifungal agents. Although greater than Amphotericin B, these statistics are an excellent starting point for improving broad-spectrum antibacterial medicines.

Conclusion

Finally, this study effectively designed, synthesized, and evaluated a novel class of antibacterial 2,5-disubstituted-1,3,4-thiadiazole derivatives. The process yielded a set of chemicals that were both effective and well-characterized. Results from biological tests demonstrate that some of these chemicals are highly effective against microbes, particularly Gram-positive ones. An electron-withdrawing substituent, particularly a nitro group at the C-5 position, is crucial for enhancing the antibacterial effect, according to the SAR analysis. The molecule with the most promise, 4f (2-(4-chlorophenyl)-5-(4-nitrophenyl)-1,3,4-thiadiazole, had significant antibacterial activity against Staphylococcus aureus, comparable to that of a conventional antibiotic. Based on the findings, the 1,3,4-thiadiazole scaffold could be used as a foundation for future antibiotic resistance-fighting medications. I. Expand the chemical library by investigating alternative C-2 and C-5 substituents. ii. Determine the therapeutic index of these compounds using mammalian cell lines. iii. Examine their in-vivo efficacy in suitable infection models. iv. Utilize molecular docking and dynamics simulations to identify the precise molecular target and understand the mechanism of action of these potent new agents.

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