



Synthesis under Microwave Irradiation of N-Substituted 3-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles and Their Anti-microbial Evaluation.

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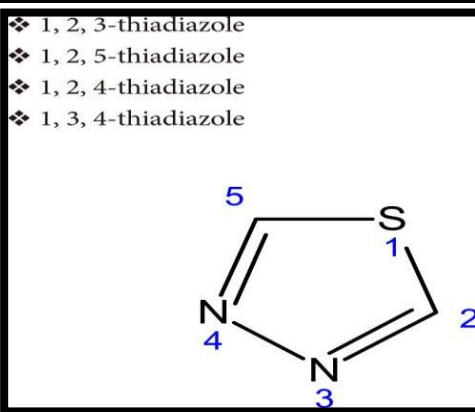
ABSTRACT

A series of N- substituted 3-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles derivatives have been synthesized by microwave irradiation method. The synthesis by using microwave assisted method gives high yields in short time. The reaction progress of the synthesized compounds was checked by TLC. The different in melting points of the synthesized compounds indicated the formation of new chemical analogues. The structures of the newly synthesized compounds were confirmed by IR and ¹H NMR spectral data. In vitro anti-microbial activity was evaluated by disc diffusion method for all the newly synthesized compounds against gram positive organisms such as Staphylococcus aureus, B.subtilis, gram negative organisms such as Escherichia coli, and fungus such as Candida albicans and Aspergillum Niger. Ciprofloxacin and Fluconazole (10µg/ml) were used as reference standard for antibacterial and antifungal activities at a concentration of 100µg/ml.

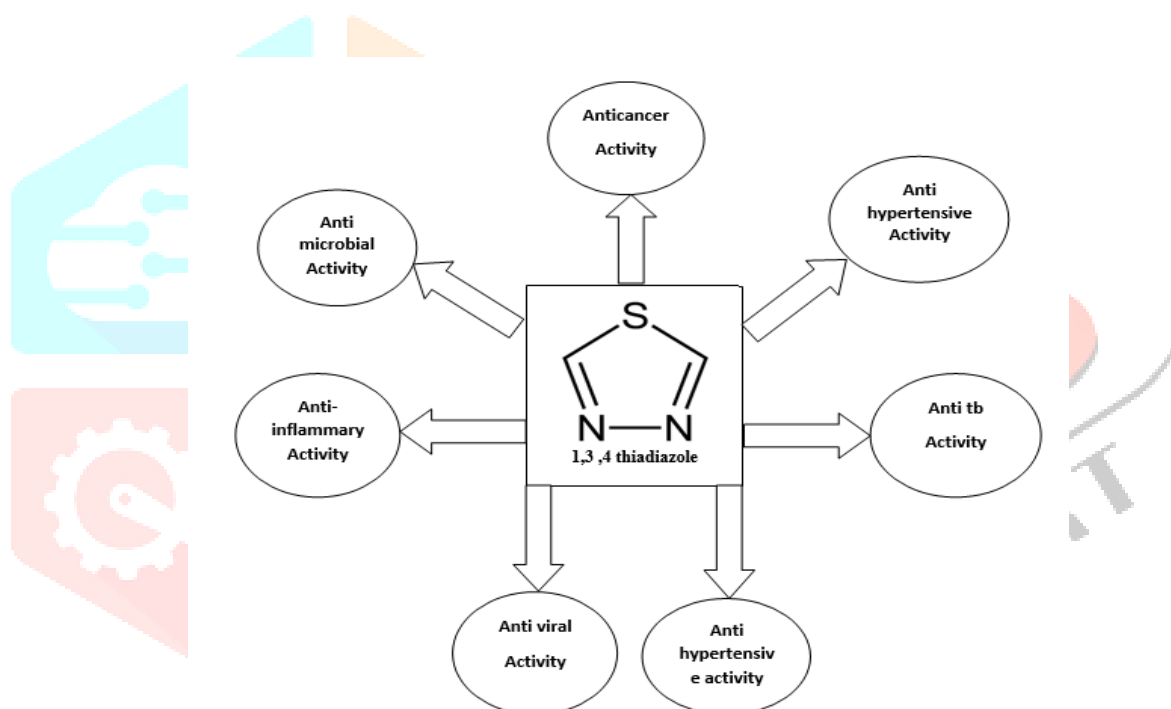
Keywords: [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles derivative, microwave irradiation and antibacterial, antifungal.

1. Introduction

Reports of the synthesis of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives have been recently published.[1-5] Thiadiazole is classified as an azole compound. These are 5-member heterocyclic compounds containing sulfur atoms with two nitrogen atoms. The presence of two double bonds gave a fragrant ring. By the name of thiadiazole, this is derived from the naming of Hanchu-Widman. Thiadiazole was first described by Fisher in 1882, but in 1890 Freud and Kuhn demonstrated the nature of the ring system. Thiadiazole and the compounds associated with their structure are called 1,3,4-thiadiazole (two nitrogen in a five-ring and one sulfur heteroatom). The structure is shown in basically, thiadiazole contains four isomers.



1,3,4-Thiadiazole is one of the most useful isomers due to its various biological effects in the body. In particular, compounds with 1,3,4-thiadiazole nuclei have a special effect on bacterial infections and anti-inflammatory effects in the body. Structurally modified derivatives of thiadiazole have been shown to have the quantity are therapeutic activities, including analgesics, antibacterial agents, anti-tuberculosis agents, anticonvulsant agents, and anti-hepatitis B virus activity. [6-8]



The biological action of thiadiazole is due to the strong aroma of the ring system, which provides high in vivo stability. Thiadiazole is a bioavailable alternative to thiazole. It is also a bioisoster of oxadiazole, oxazole and benzene. Replacing thiadiazole for this heterocyclic system increases the active lipolysis due to the increased lipophilicity of the sulfur atom. Heterocyclic compounds play an important role in bioactive organic compounds used in human and veterinary medicine as medicines or in agriculture as pesticides and insecticides. The chemical rings contained in many over-the-counter drugs have pharmacological properties or they may act as a platform for pharmacophoresis groups to interact with receptors. [9]

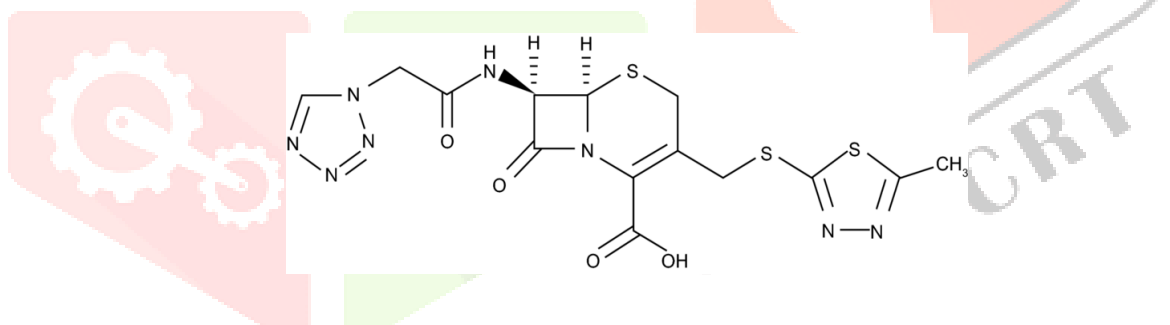
Thiadiazole belongs to the class of nitrogen-sulphur-containing heterocyclic and is widely used as a structural unit of biologically active molecules and as a useful intermediate in pharmacological chemistry. The thiadiazole ring contains several isomers (1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole). Over the past few years, substituted 1,3,4-thiadiazole derivatives have received a great deal of attention and are increasingly being studied due to their wide spectrum of pharmacological properties. Due to the presence of N-C-S-part.1,3,4-thiadiazole derivatives are thought to exhibit the quantity are biological activities. [10] Other authors believe that the biological action of

1,3,4-thiadiazole derivatives is due to the strong aroma of the ring. This provides in vivo stability for this 5-member ring system and low toxicity to high vertebrates, including humans. [11]

Microwave chemistry is the science of applying microwave radiation to chemical reactions. Microwave-assisted organic synthesis serves as the new "lead" for organic synthesis. [12-14] this technique provides a clean, simple, efficient, fast and economical one for the synthesis of many organic molecules. Such reactions have new tools in organic synthesis. The main advantages of this technology include faster reaction time and improved product production and quality.[15] Fifteen experiments have shown that the microwave method uses less solvents and sampling and extracts as compared to Soxhlet extraction. Very fast speeds have already been reported for various plant extracts.

Antibacterial agents are drugs that kill microorganisms or inhibit their growth. Thus, this microbial agent can be either a compound or any physical element. These factors effectively interfere with the growth and reproduction of pathogenic microorganisms such as bacteria, fungi, parasites and viruses. The breakthrough in the discovery of penicillin in the 1940s led to the development of an era based on rich modern antibacterial treatments. Various antibiotics and other drugs have been discovered after penicillin. The advancement penicillin, an antimicrobial, in its early 1940s became an important pillar of the era. The most important discovery since a discovery of penicillin is the pronunciation of the basic skeleton from which it was made. Thus, it has become clear that they can be chemically modified to improve their properties. In summary, antibiotics accustomed towards control accessible diseases and could be applied to treat certain medical conditions and other surgeries. [16 - 17] Based on these facts, the aim of this study was to synthesize several thiadiazole derivatives with a wide range of antibacterial substances and to inject these synthetic derivatives as an alternative to antibiotics based on their action.

All macrolides and cephalosporin contain the 1,3,4-thiadiazole group, which has strong in vitro action even against Gram-positive and Gram-negative bacteria. The best example of this is the first generation cephalosporin "Cephazoline", which was applied worldwide since the early 1970s (GlaxoSmithKline PLC, London, UK, Encef). [18-19]



Cephazoline is a parenterally administered semi-synthetic cephalosporin treatment of bacterial infections of various organs, including sepsis. [20-21] Treatments for penicillin and cephalosporin infections have often failed due to the methicillin-resistant *Staphylococcus aureus* (MRSA) strain. As a result, the use of glycopeptides antibiotics (such as vancomycin and teicoplanin) has increased. Over the years, vancomycin-resistant strains have been discovered. Several clinical trials have been initiated to compare the efficacy of cefazolin with teicoplanin and vancomycin, respectively, help stop a formation of drug resistance. The results proved that cephazolin may be the drug of choice for the prevention of surgical site infections. [22-26]

In the last two decades, the number of scientific publications on the synthesis and biological research of 1,3,4-thiadiazole has increased significantly. In addition, amine derivatives of 1,3,4-thiadiazole are being studied. Aliphatic and aromatic amines are important components of many natural or synthetic bioactive compounds. Naturally occurring amines include alkaloids such as ephedrine and pseudoephedrine. These are present in combination with many drugs and is necessary medications for its proper functioning and treatment of the nervous system. It is not surprising that more than 75% of candidate drugs contain free or substituted amine groups. [27-28]

Covalent bonds chemicals that are pharmacologically active that have similar effects but different mechanisms work so compounds can have less toxicity due to improved activity and coordinating effects.

[29] Some of the derivatives included in this review include 1,3,4-thiadiazole rings attached to different monocyclic cores. However, many of them are bis or poly heterocyclic compounds in which thiadiazole moieties are attached to other heterocyclic. In addition, this review provides some information on Structure-Activity Relationship (SAR) studies.

2. Material and Method

2.1 Chemistry

All chemicals and solvents were procured from commercial sources, purified and dried using standard procedures from literature whenever required the reagents were purchased from S. D fine, research laboratory and merck laboratory, Mumbai. The melting point of synthesized compound was determined by open capillary tube method can remain unfixed. TLC was used confirmation of reaction and the fineness of the intermediate and the final compounds by applying a single spot on TLC plate (silica gel G) using various solvents such as butanol, chloroform, water system. TLC plates were visualized under iodine chamber. IR spectra were recorded on FTIR, NMR spectra were performed in DMSO solution using Bruker 300 MHz and their chemical shift are reported in δ unit with respect to TMS as internal standard. Spectra of the mass were captured on Pesciex (model no. API 2000) software analyst 1.4.2 mode: Q1MS Q1/AUTOINJECTION from diya lab, airoli, Mumbai. General procedure for synthesis of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives are as follows:

2.2 Preparation

2.2.1 Step 1 st: - Synthesis of Substituted ethyl benzoate(a-c).

Ethyl 3-nitrobenzoate was synthesized by adding 3-nitrobenzoic acid (0.1 M) to ethanol (20 mL) and the resulting mixture was warmed for 15 to 20 min at 340 watts. a few of droplets H₂SO₄ as a catalyst. After the finishing of reaction, a solid mass was formed and checked by TLC.

2.2.2 Step 2 nd: - Synthesis of substituted benzohydrazide.

The substituted aromatic acid esters (0.1 M) were dissolved in ethanol (30 mL) and hydrazine hydrate (0.1 M) was added dropwise to the stirred mixture. The final mixture was boiled at 340 watts for 15 to 20 minutes. Excess ethanol was removed through distillation, and the substance was calmed down. Its produced crystals are filtered, thoroughly cleaned with water, then dry. TLC was utilized to ensure that the reaction was completed.

2.2.3 Step 3 rd: - Synthesis of substituted 5 – phenyl -4H-1,2,4-triazole-3-thiol.

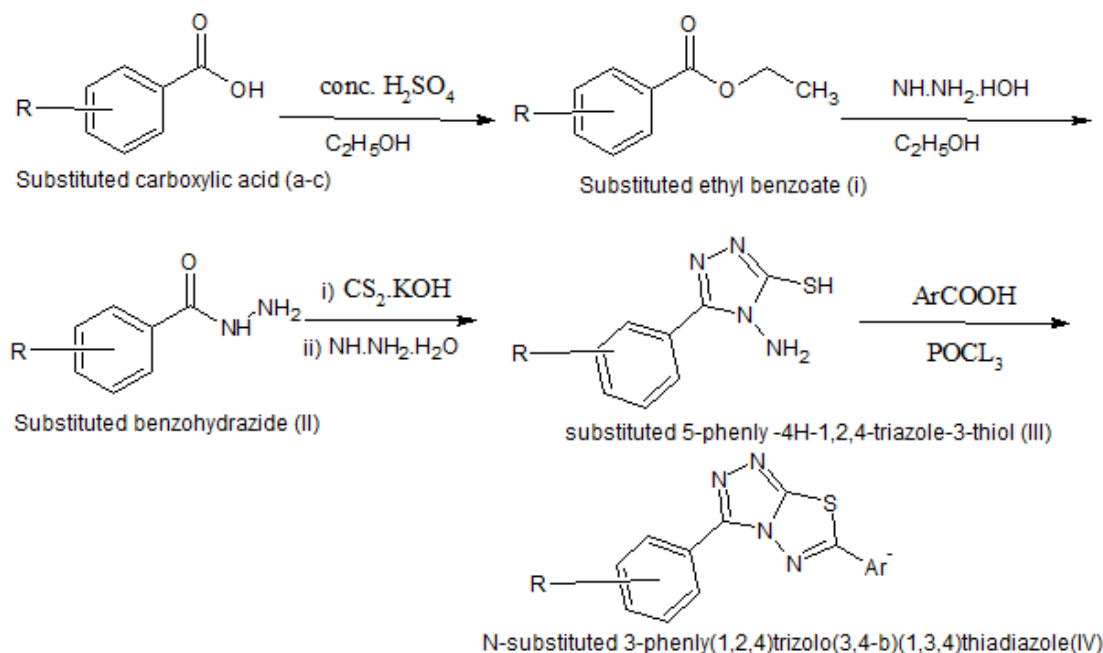
Potassium hydroxide (0.15M) was dispersible as a whole ethanol (200ml). Aryl acid hydrazide (0.1 M) was included in the above solution and the resolution was cooled in ice. To this was added carbon disulfide (0.15 M), hydrazine hydrate (15 mL, 0.3 M) with heating for 20 min for 340 watts. The combination of reactions and with H₂S gas was released. A homogeneous reaction mixture was obtained during the reaction process. The resulting mixture was mixed with (100ml) water and placed to air temp. Acidification with concentrated hydrochloric acid yields the required triazole. It was filtered, washed well using ice water and reconstituted from ethanol. Reaction completion was monitored using TLC.

2.2.4 Step 4th:-Synthesis of N-substituted 3-phenyl ([1,2,4]triazolo[3,4-b][1,3,4]thiadiazole Derivatives.

Equivalent amounts of each respective triazole (0.02M), substituted aromatic acid (0.02M) in dry phosphorus oxychloride (10ml) was heated for 20 to 25 min at 340 watts. The remaining solution was slowly stirred as it was put onto ice after reaching air temperature. To eliminate excess phosphorus oxychloride, solid sodium hydroxide and crystalline powder of sodium bicarbonate were added until the

mixture's pH reached 8. When the slurry was allowed to stand for the full night, a solid separated. It underwent filtering, a thorough cold water wash, drying, and recrystallization from hot ethanol.

REACTION SCHEME



where,

R - is substituted carboxylic acid such as 2 chloro benzoic acid, 2 bromo benzoic, 2 nitro benzoic acid acid,

Ar - aromatic acid such as P-Hydroxy benzoic acid

Scheme1: Synthetic route for the preparation of the title compound

2.3 Antimicrobial screening:

Chemicals

All chemicals and solvent were procured from commercial sources, purified and sterilized using standard procedure from literature whenever required.

2.3.1. Dilution of compound

All synthetic compounds dissolved in dimethyl sulfoxide [DMSO] Ciprofloxacin at $50 \mu\text{g} / \text{ml}$ and $100 \mu\text{g} / \text{ml}$ concentrations and the standard drug DMSO Concentration $50 \mu\text{g} / \text{ml}$ and $100 \mu\text{g} / \text{ml}$.

2.3.2. Sterilization of equipment and the chemicals

MacConkey or Medium, Normal or Medium [NO11], is disinfected in a normal saline autoclave. 150 lbs at 15 lbs pressure [121°C]. Sterilize Petri dishes, Whatman filter paper and cotton powder in the oven at 160°C for 2 hours.

2.3.3. Preparation of MacConkey agar slant

206 mg MacConkey agar medium was dissolved in 4 ml of distilled water, boiled, filled a test tube with, plugged with cotton, and sterilized in an autoclave at a pressure of 15 lbs at 121°C for 15 min. After sterilization, the tube containing the MacConkey agar medium was kept in a bent position for 30 min.

Next, fully cultured *Staphylococcus aureus* was aseptically streaked on a bent surface and cultured at a temperature of 37°C for 24 h.

2.3.4. Preparation of nutrient agar medium slant

Dissolve 112 mg nutrient or medium and 100 mg agar powder in 4 ml distilled water, boil, pour into test tube, cover with cotton and sterilize in autoclave at 15 lbs pressure (121°C) for 15 min. After disinfection, the tube containing the nutrient agar medium was kept bent for 30 min. Next, on a sloping surface, e.g. the pure cultured coli was streaked and incubated aseptically at 37°C for 24 h.

2.3.5. Preparation of suspension of test bacteria:

Using a 24-hour bacterial growth from the test bacteria, the bacterial suspension was prepared separately in sterile saline (0.85% NaCl in H₂O) to give moderate turbidity. A cloudiness in each suspension was compared with the turbidity of the solution obtained by mixing 0.5 ml 1.175% barium chloride 36N H₂SO₄ with 99.5 ml.

2.4 Method: Disc Diffusion Method

2.4.1. Preparation of culture media for antibacterial sensitivity test

MacConkey or medium (50 ml) and nutrient or medium (100 ml) were prepared due to the oblique preparation process. The autoclave was then sterilized at 15 lbs pressure (121°C) 15 minutes. After sterilization cooled down the medium to 45°C and 20 to 25 ml is placed to sterile Petri dish to thicken.

2.4.2. Inoculation of suspension of bacteria on culture media

Sterile, nontoxic swab were dipped into the standardized inoculums and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60 angles between streaking. Then the streaked inoculums is permitted in dry for 5-15 min with lid. Separately dipping clean Whatman paper discs into solutions containing synthetic drugs (50µg/ml and 100µg/ml) and the common medication ciprofloxacin (50µg/ml and 100µg/ml) in an aseptic state with assistance of sterile forceps and placed on the surface of inoculated culture media after which the plates were kept in refrigeration for 30min. for the diffusion of the compound from the paper disc into the culture media. After 30 min initial incubation of plating at 37°C for 24hrs. Each and every synthesized component (4a-c) was observed for antibacterial activity. Observation was recorded in tables then calculating its zone of inhibition in millimetre. [30-31]

3. Result and Discussion

3.1 Chemistry

Synthetic pathway for target compounds, N- substituted 3-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles, is shown in Scheme 1. The hydrazine hydrate & benzoic acid esters then treated in ethanol the produce the substituted benzoyl hydrazine which were then reacted with carbon disulfide in the presence of potassium hydroxide to produce the equivalent intermediates potassium dithiocarbazinate. This salt underwent cyclization with the excess hydrazine hydrate to give substituted 5-phenyl-4H-1,2,4-triazole-3-thiol. The resultant triazoles was modified more to N-substituted 3-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles with substituted aromatic carboxylic acids in a single pot while phosphorus oxychloride was present.

3.2 Antimicrobial activity:

Antibacterial activity of recently created molecules (1a-c) was evaluated by the disc diffusion method against *Escherichia coli*, *B. subtilis*, *Staphylococcus aureus*, and protease strains of bacteria and fungus *Candida albicans* and *Aspergillum Niger* they remained for this study. Compound code 4c was discovered to be quite effective against each the tested strains of bacteria showing the broadest spectrum of antibacterial activity when compared with standard drug ciprofloxacin and the antifungal activity when compared with standard drug Fluconazole.

Table 1: Antimicrobial screening result of synthesized compounds (1a-c) measuring the zone of inhibition in (mm)

Compound code	Zone of inhibition in(mm)							
	E. coli		B. subtilis		S. aureus		Protease	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
4a	16	13	11	8	22	18	13	11
4b	17	15	11	7	22	19	16	13
4c	21	16	15	12	23	20	17	15
Standard (Ciprofloxacin)	22	19	20	17	27	23	19	18

According to the standard medicine, compound 4c exhibits superior antibacterial activity over other compounds.

Table.2 Antifungal Screening of Compounds (1a-c)

Compound code	Zone of inhibition in(mm)			
	Aspergillum Niger		Candida Albicans	
	50µg/ml	100µg/ml	50µg/ml	100µg/ml
4a	7	8	6	8
4b	5	10	5	13
4c	9	12	15	48
Standard (Fluconazole)	11	17	20	22

Compared with standard drug the compound 4c shows better antifungal activity than other compounds.

4. Conclusion

In conclusion, a few novel N- substituted 3-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles derivatives were synthesized. The synthesis by using microwave assisted method gives high yields in short time. A number of heterocyclic azoles was produced, while its biological functions were assessed. The synthesised compounds' antibacterial activity test findings showed promise. Their structures were confirmed by elemental analysis, IR. Those chemicals' biological activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, *B.subtilis* and protease strains of bacteria and fungus *Candida albicans*, *Aspergillum niger* were used for this study by disc diffusion method. These thiadiazole derivatives had moderate to excellent antifungal and antibacterial activity, according to the findings. Particularly, among all the produced thiadiazole derivatives, compounds 4j showed significantly stronger antibacterial and antifungal activity. In order to enhance the thiadiazole derivatives' antibacterial properties, further complications are beneficial. The biological action of thiadiazole is due to the strong aroma of the ring system, which provides high in vivo stability. Thiadiazole is a bioavailable alternative to thiazolemoet. Replacing thiadiazole for this heterocyclic system increases the active lipolysis due to the increased lipophilicity of the sulfur atom.

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