2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents

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Abstract: Pathogenic microorganisms are causative agents for different types of serious and even lethal infectious diseases. Despite advancements in medication, bacterial and fungal infections continue to be a growing problem in health care. As more and more bacteria become resistant to antibiotics used in therapy and an increasing number of invasive fungal species become resistant to current antifungal medications, there is considerable interest in the development of new compounds with antimicrobial activity. The compounds containing a heterocyclic ring play an important role among organic compounds with biological activity used as drugs in human and veterinary medicine or as insecticides and pesticides in agriculture. Thiadiazoles belong to the classes of nitrogen–sulfur heterocycles with extensive application as structural units of biologically active molecules and as useful intermediates in medicinal chemistry. The potency of the thiadiazole nucleus is demonstrated by the drugs currently used. 1,3,4-Thiadiazoles and some of their derivatives are extensively studied because of their broad spectrum of pharmacological activities. The aim of this review was to highlight the main antimicrobial properties exhibited by derivatives possessing 2-amino-1,3,4-thiadiazole moiety. Many of the reported 2-amino-1,3,4-thiadiazole derivatives can be considered as lead compounds for drug synthesis, and several of them have demonstrated higher antimicrobial activity in comparison to standard drugs. Furthermore, taking into account the reactivity of the amine group in the derivatization process, 2-amino-1,3,4-thiadiazole moiety may be a good scaffold for future pharmacologically active 1,3,4-thiadiazole derivatives.

Keywords: 2-amino-1,3,4-thiadiazole, antimicrobial activity, antibacterial activity, antifungal activity, antitubercular activity, minimum inhibitory concentration

Introduction

Heterocyclic compounds play an important role among organic compounds with biological activity used as drugs in human and veterinary medicine or as insecticides and pesticides in agriculture. Chemical rings, which are present in many marketed drugs, may possess pharmacological properties or may serve as a platform for the pharmacophoric groups which will interact with the receptors.1

Thiadiazoles belong to the classes of nitrogen–sulfur containing heterocycles with extensive application as structural units of biologically active molecules and as useful intermediates in medicinal chemistry. There are several isomers of the thiadiazole ring (eg, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole and 1,3,4-thiadiazole; Figure 1). During the past years, substituted 1,3,4-thiadiazole derivatives have received significant attention and have been increasingly investigated due to their broad spectrum of pharmacological properties. It is supposed that 1,3,4-thiadiazole derivatives exhibit various biological activities due to the presence of =N–C–S= moiety.2 Other authors assume that the biological activities of 1,3,4-thiadiazole derivatives are due to
the strong aromaticity of the ring, which also provides great in vivo stability to this five-membered ring system and low toxicity for higher vertebrates, including human beings.¹

There are some studies that show the importance of isosterism for the pharmacological profile of a compound. According to these studies, 1,2,4-thiadiazole is the bioisostere of pyrimidine, while the 1,3,4-thiadiazole is the bioisostere of pyridazine through the substitution of –CH=CH– by –S– (Figure 2).⁴,⁵ The thiadiazole ring is also a bioisostere of oxadiazole, oxazole, thiazole and benzene ring.⁴,⁶ The bioisosteric replacement of a ring with another ring might lead to compounds with increased lipophilicity and improved biological properties. The thiadiazole derivatives, due to the presence of sulfur atom that gives high liposolubility, show oral absorption and good cell permeability leading to a good bioavailability. In addition, substitution of a homocyclic ring with a heterocycle makes the synthesis of different analogs possible which interact more with the receptors.⁴,⁷,⁸

Considering the high prevalence of pyrimidine derivatives in nature and also the presence of pyridazine ring in compounds possessing pharmacological activities (eg, the antidepressant, minaprine; GABA-A antagonist, gabazine; nonsteroidal anti-inflammatory drug, emorfazone and the antibacterial cephalosporin, cefozopran), the potential of thiadiazole derivatives to exhibit biological activities is very high.⁹–¹¹

Moreover, 1,3,4-thiadiazole derivatives can produce mesoionic salts (Figure 3). Mesoionic system contains a pentatomic heterocyclic ring which possesses a sextet of $p$ and $\pi$ electrons and positive charge counterbalanced by formal negative charge. Despite their internal charges, the mesoionic compounds are neutral and able to cross cellular membranes, and this contributes to the good cell permeability of 1,3,4-thiadiazole derivatives. The mesoionic nature of 1,3,4-thiadiazoles enables these compounds to interact strongly with biomolecules (eg, DNA and proteins).⁹,¹²

The biological importance of 1,3,4-thiadiazole derivatives has been reported following the discovery of heterocyclic sulfonamides as reasonable antimicrobial agents (eg, sulfathiazole 1; Winthrop Chemical Company, NY, USA, 1940).¹³,¹⁴ In analogy to sulfathiazole, other sulfonamides showing similar activity such as “sulfamethizole” 2 (4-amino-$N$-(5-methyl-1,3,4-thiadiazol-2-yl)benzene sulfonamide, Rufol [Urgo Laboratories, Chenove, France])¹⁵,¹⁶ or “sulfaethidole” 3 (4-amino-$N$-[5-ethyl-1,3,4-thiadiazol-2-yl]benzene sulfonamide, Globucid [Schering, Berlin, Germany])¹⁷ were prepared. Except sulfathiazole that is still used in the treatment of Haemophilus vaginalis vaginitis,¹⁸ sulfamethizole and sulfaethidole currently possess only historical importance.

![Figure 1](image1.png)  
**Figure 1** Natural isomers of the thiadiazole ring.

![Figure 2](image2.png)  
**Figure 2** Isomerism between the six-membered diazaheterocycles and thiadiazole derivatives.

![Figure 3](image3.png)  
**Figure 3** Chemical structure of the mesoionic salt derivatives formed by 1,3,4-thiadiazole compounds.
The synthesis of “acetazolamide” 4 (5-acetylamino-1,3,4-thiadiazol-2-sulfonamide) by Roblin and Clapp19 (Lederle Laboratories, Pearl River, NY, USA) as carbonic anhydrase inhibitor reoriented the researchers to sulfonamides bearing 1,3,4-thiadiazole ring. Acetazolamide was marketed as Diamox (Lederle Laboratories, American Cyanamid Company, Pearl River, NY, USA) in 1954.19–21 Although its use as a diuretic is limited by systemic acidosis as a side effect,22 acetazolamide is currently a good antiglaucoma agent. Its methylated derivative, “methazolamide” 5 (Neptazane [Fera Pharmaceuticals, Locust Valley, NY, USA]), is a more potent carbonic anhydrase inhibitor and displays diuretic, antiglaucoma and potential antineoplastic activity.23

Olsen et al24 discovered the cytostatic properties of “2-amino-1,3,4-thiadiazole,” having as a result many publications that launched new 1,3,4-thiadiazole derivatives with potential anticancer activity.

The synthetic studies concerning the therapy of parasitic infections gave “megazol” 6 (2-amino-5-[1-methyl-5-nitro-1H-2-imidazolyl]-1,3,4-thiadiazole, CL 64855),31 a nitroimidazole extremely active in experimental infections caused by Trypanosoma cruzi and Trypanosoma brucei as well as drug-resistant forms of trypanosomiasis. Megazol has been found to be the best-known anti-trypanosomal candidate for the treatment of sleeping sickness in Africa and Chagas disease in South America. However, due to the high toxicity (mutagenic and genotoxic properties), its development has been postponed, but it serves as a lead compound for the development of new anti-trypanosomal agents.26–31

1,3,4-Thiadiazole ring is the constitutive part of some cephalosporins and cephamycins that showed high in vitro activity against both Gram-positive and Gram-negative bacteria. A good example is “cefaclor” 7, a first-generation cephalosporin which has been used worldwide since the early 1970s (GlaxoSmithKline plc, London, UK; Ancef).32,33

Cefazolin is a semisynthetic cephalosporin for parenteral treatment of bacterial infections in various organs, including septicemia.32–34 The treatment of infections with penicillins and cephalosporins failed many times due to the methicillin-resistant Staphylococcus aureus (MRSA) strains. As a result, the use of glycopeptide antibiotics (eg, vancomycin and teicoplanin) was increased. During the past years, vancomycin-resistant strains were found. To prevent the development of drug resistance, several clinical studies to compare the efficacy of cefazolin vs teicoplanin and vancomycin, respectively, were initiated. The results proved that, for the prevention of surgical-site infections, cefazolin might be the first-line medication.34–38

1,3,4-Thiadiazole ring is an important scaffold known to be associated with several biological activities including antimicrobial,39–41 antituberculosis,42 antiviral,43 analgesic,44 antidepressant and anxiolytic,45 antihypertensive,46 anti-convulsant, anti-inflammatory,47,48 local anesthetic49 and kinesin inhibitors.50,51 During the past 2 decades, the number of scientific publications concerning the synthesis and biological investigation of 1,3,4-thiadiazoles has considerably increased. In addition, the amine derivatives of 1,3,4-thiadiazole are also studied. Aliphatic and aromatic amines are important moieties in many natural or synthetic biologically active compounds. Naturally occurring amines include alkaloids such as ephedrine and pseudoephedrine, which are present in several drug combinations, and catecholamine neurotransmitters (dopamine, epinephrine and norepinephrine) are essential for the proper activity of the nervous system and valuable drugs used in therapy. It is not surprising that more than 75% of drug candidates contain free or substituted amine groups.52,53

The cytostatic properties of 2-amino-1,3,4-thiadiazole and the anti-trypanosomal properties of megazol are evidence of the biological potential of 2-amino-1,3,4-thiadiazole moiety. The aim of this review was to highlight the main antimicrobial properties exhibited by derivatives possessing 2-amino-1,3,4-thiadiazole moiety. Furthermore, taking into account the reactivity of the amine group in the derivatization process, 2-amino-1,3,4-thiadiazole moiety
may be an excellent scaffold for future pharmacologically active 1,3,4-thiadazole derivatives. The covalent bonding of biologically active compounds with similar effect but acting by different mechanisms could lead, by synergism, to compounds with improved activity and less toxicity. Some of the derivatives included in the review have the 1,3,4-thiadiazole ring linked to different homocyclic cores but many of them are bis- or polyheterocyclic compounds bearing the thiadiazole moiety linked to other heterocyclic rings. In addition, this review provides some information about structure–activity relationship (SAR) studies.

Antimicrobial activities associated with 2-amino-1,3,4-thiadiazole system
Pathogenic microorganisms are causative agents for different types of diseases such as upper and lower respiratory tract infections, typhoid fever, gastrointestinal infections, gynecological infections, sexually transmitted diseases, urinary tract infections, bacterial meningitis, osteomyelitis and malaria and also for severe diseases such as tuberculosis, influenza, syphilis and acquired immunodeficiency syndrome (AIDS). Taking into account that infectious diseases affect millions of people and cause many deaths worldwide, it can be said that anti-infective agents have saved more lives than other classes of drugs.

Antibacterial and antifungal activities
The antimicrobial chemotherapy is the way to combat infections through the pharmacological effects of the drugs used. Antimicrobial agents have specific toxic action on pathogenic organisms. Since the introduction of the first antibiotic (penicillin, 1942) into medical practice, there has been an ongoing race between scientists and pathogenic bacteria. In the struggle for existence, the microorganisms constantly adapt by selecting higher invasive and more resistant strains. Despite the large number of antibiotics and chemotherapy agents available for medical use, the bacterial infections have dramatically increased due to bacterial resistance to antimicrobial drugs. On the other hand, the spread of HIV infection combined with the increased use of powerful immunosuppressive drugs for cancer therapy and organ transplants led to increased incidence of fungal infections among immunocompromised patients. Although most fungal infections were superficial in the past, the incidence of systemic fungal infections has currently increased. The gravity of bacterial and fungal infections became a major worldwide problem, and the World Health Organization chose the antimicrobial resistance as the theme of the 2011 World Health Day. Due to the occurrence of bacterial resistance (eg, MRSA that is resistant to many antibiotics), researchers are in a continuous effort to counteract infections by synthesizing new effective antibacterial and antifungal agents.

Thiadiazole ring acts as a pharmacophore. It is also a bioisostere of the thiazole ring included in the third- and fourth-generation cephalosporins, and this observation makes it possible to use it in the synthesis of antimicrobial agents. Upadhyay and Mishra synthesized the 5-(4-substituted phenyl)-1,3,4-thiadiazol-2-amine derivatives and performed in vitro antibacterial activity against S. aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa and antifungal activity against Aspergillus niger and Candida albicans by disk diffusion technique. Fluorinated and chlorinated compounds showed good inhibitory effects on S. aureus and B. subtilis. In addition, halogenated compounds showed moderate inhibitory effects for E. coli and P. aeruginosa. Significant antifungal activity against A. niger and C. albicans was exhibited by derivatives bearing oxygenated substituents at phenyl ring (inhibition between 58% and 79%) with MIC values of 24–26 μg/mL (controlled to ciprofloxacin, MIC=18–20 μg/mL) for S. aureus and B. subtilis. Resistance as the theme of the 2011 World Health Day. Due to the occurrence of bacterial resistance (eg, MRSA that is resistant to many antibiotics), researchers are in a continuous effort to counteract infections by synthesizing new effective antibacterial and antifungal agents.

A new series of 4-amino-2-[5-{(4-substituted phenyl) amino}-1,3,4-thiadiazol-2-yl]phenols was synthesized, and the compounds were in vitro evaluated for their antimicrobial activity. Among the series, the chlorinated and fluorinated derivatives exhibited good antibacterial activity against S. aureus and E. coli strains and antifungal activity against A. niger with MIC values of 25 μg/mL.
Other fluorinated compounds incorporating 1,3,4-thiadiazole ring and the diflunisal (a nonsteroidal anti-inflammatory drug) structure were tested for antimicrobial activity using microwell dilution assay and MIC agar dilution assay. 5-(2′,4′-Difluoro-4-hydroxybiphenyl-5-yl)-phenylamino-1,3,4-thiadiazole 10 showed activity against *E. coli* and *Streptococcus pyogenes* at a concentration of 31.25 μg/mL (inhibition zone of 16–18 mm), two times greater than that observed for the control drug, ofloxacin (MIC = 62.5 μg/mL). In addition, another standard drug, cefepime, was found less active against the bacteria mentioned earlier.

Kadi et al.54,65 have synthesized new 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazole derivatives 11. The investigation of antimicrobial screening of the synthesized compounds revealed that the best antibacterial activity was exhibited by the p-chlorophenyl 11c and p-nitrophenyl 11e derivatives against Gram-positive microorganisms such as *B. subtilis* and *S. aureus*. Only p-nitrophenyl derivative 11e showed good activity against the Gram-negative bacteria *E. coli* in comparison to the reference drug, ampicillin. SAR studies have shown that the introduction of another adamantyl moiety on C-5 of thiadiazole ring increased the antifungal activity against *C. albicans*.

The in vitro antimicrobial activity of some new 1,3,4-thiadiazole derivatives such as 12 having a D,L-methionine moiety has been evaluated against several bacterial strains by Pintilie et al.66 From the findings, the authors revealed that 1,3,4-thiadiazole derivatives have good activity against *Bacillus anthracis* and *Bacillus cereus*, the most active compound 12c possessing a 4-methylphenyl moiety on the heterocyclic ring. The compounds showed a very weak activity against *S. aureus* and *E. coli* strains and were inactive against *Sarcina lutea* strain. The antimicrobial activity of some thioethers derived from 2-amino-5-mercapto-1,3,4-thiadiazole was found to be dependent on the substitution at the mercapto group. The unsubstituted and halogenated aryl derivatives 13 proved to be the most active compounds against *Salmonella typhimurium* and *C. albicans*. 67

A series of 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole derivatives such as 14 synthesized by Matysiak and Malinski68 has been evaluated for antifungal activity against *C. albicans* and *Candida nonalbicans* species. Many derivatives exhibited higher activity against *C. nonalbicans* species compared to standard drugs, itraconazole (MIC = 85.6 μg/mL) and fluconazole (MIC = 120.8 μg/mL), 2,4-dichlorophenylamino derivative 14d (MIC = 37.8 μg/mL) and morpholinoethylamino
derivative 14f (MIC =34.4 µg/mL) being the most active compounds. The aminoderivatives substituted with the methyl, phenyl, halogenophenyl, ethoxyphenyl and morpholinoalkyl groups showed higher antifungal activity against C. albicans strains. For some of them, such as phenylamino derivative 14b (MIC =36.3 µg/mL) and 2,4-dichlorophenylamino derivative 14d (MIC =32.6 µg/mL), an activity higher than that of itraconazole (MIC =47.5 µg/mL) was observed.

A number of 2-amino-1,3,4-thiadiazoles substituted at C-5 of thiadiazole ring with the phenyl or phenol group showed moderate to significant antibacterial and antifungal activities against Gram-positive bacteria (S. aureus and B. cereus), Gram-negative bacteria (E. coli and P. aeruginosa) and fungal strains (A. niger and Aspergillus fumigatus) compared to standard drugs, streptomycin and fluconazole. The compounds 15 possessing the p-nitroaniline moiety showed the most promising antibacterial and antifungal properties.

Having 5-(4-chlorophenylamino)-2-mercapto-1,3,4-thiadiazole as starting material, Sah et al have synthesized the Mannich base which was converted to the corresponding formazans with aromatic, benzenesulfonic acid or sulfonamide moiety. Among the synthesized compounds, the aromatic formazans 18 were the most active compounds showing good activity (zone of inhibition of 15–19 mm) against Salmonella typhi strain (compound 18a) and E. coli strain (compound 18b) at the concentration of 500 µg/disk. The same compounds showed good antifungal activity against A. niger and/or Penicillium sp. and only moderate activity against C. albicans.

Dogan et al have examined the effect of various substitutions at the amine group of 2-amino-5-(3-hydroxy-2-naphthyl)-1,3,4-thiadiazole on antimicrobial activity against different bacterial and fungal strains. Among the series, the p-chlorophenyl derivative 19 was marginally active against S. aureus (MIC value of 62.5 µg/mL), and it may be considered for the development of new antibacterial agents.

Insertion of a long alkenyl/hydroxyalkenyl chain to the fifth position of 1,3,4-thiadiazole ring is a useful tool in the
synthesis of biologically active compounds. Such 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 20 showed good antibacterial activity against *E. coli, S. aureus, P. aeruginosa, S. pyogenes* and *Klebsiella pneumoniae* and moderate to good antifungal activity against *C. albicans, A. fumigatus, Penicillium marneffei* and *Trichophyton mentagrophytes*. Antimicrobial activity data indicated that derivatives 20a and 20b having a hydroxyalkenyl chain substituent at C-5 are good antibacterial agents with almost equal potency against *K. pneumoniae* as the reference drug, chloramphenicol. The position of the hydroxyl group did not have a significant influence on the pharmacological effect. The alkenyl derivatives such as 20c were found to be potent antifungal agents compared to standard drug, griseofulvin.74

2-Amino-5-R-1,3,4-thiadiazole derivatives have also been investigated for their ability as complexation agents to obtain compounds with enhanced biological properties due to the presence of metal ions. Thus, 5-(2-aminoethyl)-2-amino-1,3,4-thiadiazole 21 and its Cu(II) and Ni(II) complexes 22a-b were reported by Barboiu et al.75 The antifungal activity of the ligand and its metal complexes against two *Aspergillus* species and *C. albicans* was investigated. Compared to antifungal drug, clotrimazole, the ligand was much less active, while the metal complexes showed an increased antifungal activity. The most active compound, Cu(II) complex 22a, was almost as effective as clotrimazole against *A. niger* and more effective against *Aspergillus flavus*. Obaley et al76 have synthesized 2,5-diamino-1,3,4-thiadiazole 23 as a bioactive ligand and its metal complexes with biologically important ions, such as Co(II), Ni(II) and Cu(II). The ligand behaved as tridentate neutral ligand which coordinated to the metal ions via sulfur and nitrogen of the amines giving solid chelates with general formulae [(ML₂)Cl₂] and octahedral geometric structures. The in vitro antimicrobial study of the ligand and its metal complexes showed the antibacterial activity of the tested compounds and no antifungal activity against *A. niger* and *Penicillium marneffei* sp. The most sensitive microorganism to ligand and its metal complexes was *Neisseria gonorrhoeae*. The metal complexes showed comparable or greater activity against some of the tested microorganisms compared to the ligand. *S. typhi, Shigella* sp. and *P. aeruginosa* were more sensitive to the metal complexes than *S. aureus, Klebsiella* sp. and *E. coli*. In addition, the metal complexes did not show toxicity against the activity of some kidney, liver and serum enzymes, making these compounds promising candidates for antibacterial treatment.

Taking into account that the presence of a heterocyclic ring increases the probability of antimicrobial properties of a compound, bis 1,3,4-thiadiazole derivatives such as 24 have been synthesized from various dicarboxylic acids and were investigated for in vitro antibacterial and antifungal activities. The results showed that the tested compounds exhibited comparable antimicrobial activity with the standard antibiotics such as ciprofloxacin and griseofulvin, the most active compound being the methyl derivative (n = 1).77 Moreover, Salimon et al.41 described the synthesis of 2,5-di-[5-amino-1,3,4-thiadiazol-2-thiomethyl]-1,3,4-thiadiazole 25 through the reaction of 2,5-dimercapto-1,3,4-thiadiazole with chloroacetic acid and
thiosemicarbazide. The antimicrobial screening revealed that the tris-1,3,4-thiadiazole derivative displayed good antimicrobial activity against the Gram-positive bacteria *S. aureus* and *Corynebacterium diphtheriae* and yeasts such as *Saccharomyces cerevisiae* and weak activity against Gram-negative bacteria. Other tris-2,5-disubstituted 1,3,4-thiadiazole derivatives such as 26 synthesized by Rezki et al.\(^\text{P9}\) showed good antibacterial activity against Gram-positive (*Streptococcus pneumoniae, B. subtilis, S. aureus*) and Gram-negative bacteria (*P. aeruginosa, E. coli, K. pneumoniae*) and antifungal activity against *A. fumigatus, C. albicans, Geotrichum candidum* at concentrations ranging between 8 and 31.25 μg/mL.

The substitution of the amine group decreased the activity in the following order: methyl > ethyl > phenyl.

Demirbas et al.\(^\text{P11}\) have synthesized several 1-(5-arylamino-[1,3,4]thiadiazol-2-yl)methyl-4-amino-3-R-5-oxo-4,5-dihydro-[1,2,4]triazoles 29 and 30 which exhibited antimicrobial properties on different microbial strains. *p*-Chlorophenyl derivative 29d and *p*-methoxyphenylamino derivative 30 showed the most potent antimicrobial activity against the tested microorganisms. For example, compound 30 showed promising antimicrobial activity against all the bacterial strains: *E. coli, Yersinia pseudotuberculosis, P. aeruginosa, Enterococcus faecalis, S. aureus, B. cereus* (diameter of the inhibition zone ≥20 mm) with the best results against *E. coli* and *P. aeruginosa* (diameter of the inhibition zone ≥30 mm). Methyl derivative 29a showed good activity against *E. coli, P. aeruginosa, K. pneumoniae* and *B. subtilis* strains (diameter of the inhibition zone ≥17 mm). Benzyl derivative 29b showed good activity against *K. pneumoniae* (diameter of the inhibition zone ≥17 mm) and moderate activity against *E. coli, S. aureus* and *B. subtilis* strains (diameter of the inhibition zone 11–16 mm), while phenyl derivative 29c exhibited moderate activity against *K. pneumoniae* (diameter of the inhibition zone 11–16 mm) and antifungal activity against *Penicilloium* sp. (diameter of the inhibition zone 20 mm).

An attempt to prepare active compounds of the thia diazole derivative series combining the xylosyl moiety and the aryltetrazole ring gave compound 27 exhibiting *S. aureus* inhibitory activity.\(^\text{P9}\) It is well known that furan derivatives bearing a nitro group in the 5-position and a group of the aryltetrazole ring gave compound 28

The substitution of the –NH– group in arylamino unit by a hydrophobic methyl group decreased the antimicrobial activity.\(^\text{P32,33}\)

Serban et al.\(^\text{P10}\) prepared 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole 28 with antibacterial activity in animals after oral or intramuscular administration. The free amine group conferred the maximum antibacterial activity, while the substitution of amine group decreased the activity in the following order: methyl > ethyl > phenyl.
Following the discovery of the natural antimicrobial pyrazole C-glycoside, pyrazofurin, much attention has been given to the pyrazole derivatives. Compounds containing pyrazole moiety attached to the 1,3,4-thiadiazole ring have been synthesized by Bekhit et al. Among the studied compounds, the tolyl derivative showed about half of the activity of ampicillin against Gram-positive microorganism *S. aureus*. Even if the antimicrobial activity is not very high, the complexity of the reported compounds makes them promising pharmacological agents. In the search for new antimicrobial agents, 2-amino-1,3,4-thiadiazole derivatives bearing isomeric pyridyl ring have been synthesized and the antibacterial studies have also been reported. The naphthyl derivative exhibited very weak inhibitory activity against *S. aureus* and *E. coli*, while the benzyl derivative was more active than other studied compounds against tested Gram-positive and Gram-negative bacterial strains.

Similar hybrid compounds which have a 2-pyridyl ring linked to 1,3,4-thiadiazol moiety through a methylene group (e.g., compound 34) also exhibited moderate activity against some Gram-positive cocci, Gram-negative rods and fungi. Some 1,3,4-thiadiazole derivatives such as 35 bearing pyrimidine ring have been tested for in vitro antifungal activity against *E. coli* and *Rhizobium* spp. Opposite to what was observed in the previous examples, the pyridyl derivatives 35e-f exhibited the best inhibition activity among the tested compounds.

Andrews et al. synthesized some new 2-amino-1,3,4-thiadiazole derivatives substituted at C-5 of thiadiazole ring with dihydropyrimidine moiety. The compounds such as 36 were evaluated for in vitro antibacterial activity at a concentration of 10 μg/mL by measuring the inhibition area on agar plates (diffusion method). Moderate activity against *E. coli* was shown by nitro derivatives 36c-d using dimethyl sulfoxide (DMSO) as a control and ciprofloxacin as standard drug. Hydroxyl derivatives 36a-b exhibited moderate to good inhibitory activity against *P. aeruginosa*, *S. aureus* and *E. coli*.

New 2-[4-(4-bromophenyl)-5-(2-furyl)-4H-1,2,4-triazole-3-yl]mercaptomethyl-5-alkyl/arylamino-1,3,4-thiadiazoles 37 were synthesized and have been evaluated for in vitro antifungal activity against *Microsporum gypseum*, *Microsporum canis*, *T. mentagrophytes*, *Trichophyton rubrum* and...
C. albicans. Both types of compounds bearing aliphatic and aromatic substituents inhibited the growth of fungal strains at concentrations ranging between 4 and 8 μg/mL, the most susceptible microorganism being T. mentagrophytes (MIC value of 4 μg/mL).

Onkol et al\textsuperscript{91} have synthesized several 2-[(1(2H)-phthalazinone-2-yl)methyl(ethyl)-5-arylamino-1,3,4-thiadiazole derivatives such as 38. The antimicrobial studies showed that the compounds are active against B. subtilis strains and two yeast-like fungi: C. albicans and Candida parapsilosis. Even if the antimicrobial activity was lower compared to the standard drugs, ampicillin and fluconazole, the tested compounds could be good starting materials for better antibacterial and antifungal agents. A number of 1-[1,3,4-thiadiazol-2-yl]-3-methylthio-6,7-dihydrobenzo[c]thiophen-4(5H)ones 39 have been evaluated for antimicrobial activity against several bacterial and fungal strains. The compounds exhibited antifungal activity against C. albicans and high potency against B. subtilis. The presence of a bulky group increased the antimicrobial activity. Thus, p-nitrophenyl derivative 39d had the same potency as nitrofurantoin against B. subtilis strains.\textsuperscript{92}

Siddiqui and Alam\textsuperscript{93} and Siddiqui et al\textsuperscript{94} have synthesized some new 5-(1H-indol-3-ylmethyl)-N-(substituted phenyl)-1,3,4-thiadiazol-2-amine derivatives such as 40. The antibacterial and antifungal studies showed good to high activity against E. coli and C. albicans, respectively, for some of the analyzed compounds.

Vasoya et al\textsuperscript{95} reported the synthesis and antimicrobial screening of new 2-(3′-chboro-5′-phenoxybenzo[b]thiophen-2′-yl)-5-arylamino-1,3,4-thiadiazole derivatives such as 41. Upon the evaluation of antimicrobial activity, it has been found that almost all compounds exhibited antimicrobial activity against E. coli, Bacillus megaterium, S. aureus or A. niger at a concentration of 40 μg/mL. The activity was comparable or higher than the reference drugs such as benzylpenicillin, amoxicillin, ciprofloxacin, erythromycin and griseofulvin. The findings revealed that antimicrobial activity was diminished by the introduction of the
Amir et al. have synthesized new 2-R-5-(6'-chloro-1',3'-benzo[d]thiazol-2-yl-amino)-1,3,4-thiadiazoles 45, and the antimicrobial activity has been evaluated. The compounds having 2-acetoxyphenyl (45a), 2-naphtylmethyl (45b) and 2,4-dichlorophenoxymethyl (45e) groups showed potent antifungal activity. New 1,3,4-thiadiazole derivatives such as 46 incorporating a fluorobenzothiazole moiety have been synthesized by Vedavathi et al. The antimicrobial screening showed significant antibacterial and antifungal activity for some of the studied compounds (eg, morpholine and piperazine derivatives).

Other chlorobenzothiophene derivatives bearing the N-imidazolylthiadiazolylamine moiety were investigated for their antimicrobial activity. The benzimidazole derivative 42 showed marked antifungal activity against A. niger, while the 4,5-dihydroimidazol derivative 43 showed moderate antibacterial activity against B. cereus.

2-(3',5'-Dichlorobenzo[b]thiophen-2'-yl)-5-arylamino-1,3,4-thiadiazole derivatives 44 exhibited antimicrobial activity against bacterial and fungal strains. The SAR study showed that the arylamino substituent at C-5 of the thiadiazole ring can change the antimicrobial spectrum. Thus, the nitro derivative 44c showed activity against S. aureus similar to benzylpenicillin, the o-tolylamino derivative 44a was as active as amoxicillin against Gram-negative bacteria E. coli and Proteus vulgaris, while the p-tolylamino derivative 44b exhibited antifungal activity against A. niger.

1,3,4-Thiadiazole derivatives 47, 48 and 49 bearing benzimidazole (47), benzoxazole (48) and benzothiazole (49) moiety, respectively, were screened for antibacterial activity against E. coli and Bacillus cirrhosis and antifungal activity against A. niger and Penicillium worthmanni. In comparison to norfloxacin and griseofulvin, all the tested compounds exhibited moderate to good antibacterial and antifungal activities at a concentration of 100 μg/mL. It should be noted that pyridine-substituted compounds 47b, 48b and 49b were more active at every concentration (100, 50 and 25 μg/mL) than benzyl-substituted compounds. Among the three reported heterocyclic systems, the benzimidazole derivatives such as 47 have shown better antimicrobial activity.

R = H (a); 3-Cl (b); 4-Cl (c); 4-CH₃ (d); 4-CH₂O (e); 2-NO₂ (f)
The synthesis of new polymers derived from poly(vinyl chloride) (PVC) was carried out by Yousif et al.\textsuperscript{100} The modified PVC polymers\textsuperscript{50} contain 1,3,4-thiadiazole ring and were obtained through the chlorine displacement reaction from PVC with heterocyclic moiety. The preliminary screening for the antimicrobial activity of the polymers was performed on three bacterial strains: \textit{S. aureus}, \textit{Streptococcus viridans} and \textit{E. coli} and three fungal strains: \textit{Fusarium oxysporum}, \textit{Alternaria alternata} and \textit{Alternaria solani} with promising results.

8-Hydroxyquinoline derivatives, such as nitroxoline and chlorquinaldol, are well-known antimicrobial agents used for the treatment of urinary and intestinal infection, respectively. A new series of metal chelates of 5-[4-chlorophenyl-(1,3,4-thiadiazol)-2-ylaminomethylene]-8-hydroxyquinoline derivative\textsuperscript{51} was synthesized by Patel and Singh.\textsuperscript{101} The antifungal activity of the ligand and its transition metal chelates were evaluated. The findings suggested that the chelates are more toxic compared to the ligand. Cu\textsuperscript{2+} chelate\textsuperscript{51a} was the most active among the studied compounds.

The in vitro activity of 1,3,4-thiadiazole derivatives\textsuperscript{52} bearing an imidazo[2,1-b]thiazole moiety has been evaluated against bacterial strains of \textit{S. aureus}, \textit{P. aeruginosa} and \textit{E. coli} and fungal strains of \textit{C. albicans}, \textit{C. parapsilosis}, \textit{Candida krusei}, \textit{T. mentagrophytes}, \textit{Trichophyton tonsurans} and \textit{M. gypseum}. The ethyl derivative\textsuperscript{52b} showed the highest activity against \textit{E. coli}, and both methyl (\textsuperscript{52a}) and ethyl (\textsuperscript{52b}) derivatives were very active against \textit{T. tonsurans}.\textsuperscript{102}

Hybrid molecules\textsuperscript{53} incorporating a fluoroquinolone moiety and the thiadiazole ring have been synthesized, and the antimicrobial activity was investigated. The screening data revealed that the tested compounds showed moderate to excellent antibacterial and antifungal activity. Thus, the phenyl derivative\textsuperscript{53a} was more active against \textit{A. niger} than nystatin, and the tolyl derivative\textsuperscript{53b} was more active against \textit{P. aeruginosa} than chloramphenicol. Compared to the known antibacterial fluoroquinolones, the hybrid compounds\textsuperscript{53a-b} showed a very narrow antimicrobial spectrum.\textsuperscript{103}

The hybrid derivatives\textsuperscript{54} having the 1,3,4-thiadiazole ring attached to the antipyrine moiety showed weak inhibitory activity against Gram-positive bacteria \textit{S. aureus} and \textit{B. subtilis} and no activity against some Gram-negative bacteria.\textsuperscript{104} However, the introduction of a third heterocyclic nucleus in the structure of some hybrid antipyrine derivatives gave the compounds such as\textsuperscript{55} with very good antimicrobial activity against enteric bacteria such as \textit{E. coli}, \textit{Enterobacter aerogenes} and \textit{Y. pseudotuberculosis} and Gram-negative bacteria \textit{P. aeruginosa} (inhibition zone >20 mm at 5 μg/mL). The most active derivative\textsuperscript{55a} was two times more active than ampicillin.\textsuperscript{105}
Hybrid sulfonamide-1,3,4-thiadiazole derivatives such as 56, combining two pharmacologically active molecules in one new compound, have been synthesized by Camoutsis et al.\textsuperscript{106} The antimicrobial study against Gram-positive bacteria, Gram-negative bacteria and fungi has revealed some differences depending on the nature of the substituents. The relationships between the structure and the antimicrobial activity showed that pyrrolidine, piperidine and methylpiperazine derivatives are more active, the pyrrolidine derivative 56a exhibits the best antibacterial activity and the methylpiperazine derivative 56c exhibits the best antifungal activity. Moreover, the SAR study showed that the inhibitory effect depends on the substitution at the phenylamino group. Thus, substitution at the para position of the benzene ring with the CF\textsubscript{3} group or chlorine improved the antimicrobial activity which indicates that the antimicrobial activity is correlated with the lipophilicity of the compounds expressed by the calculated values of ClogP.

Hybrid coumarin-1,3,4-thiadiazole derivatives such as 57 have been synthesized by Serban et al.\textsuperscript{107–109} and the antimi-
crobial activity has been investigated. The preliminary results of antimicrobial activities indicated that some compounds exhibited moderate to good antibacterial and antifungal activity with a narrow antimicrobial spectrum limited to P. vulgaris, P. aeruginosa and C. albicans. Except the 3-[5-(4-
methylphenylamino)-1,3,4-thiadiazol-2-yl]-6-nitrocoumarin derivative 57h, which had no activity against the bacterial and fungal strains, all the tested compounds showed good activity against P. aeruginosa at a concentration of 50 \( \mu \)g/disk (inhibition zone of 19–22 mm), which indicates that the coumarin-1,3,4-thiadiazole system might be a promising scaffold for new antibacterial agents. In addition, the introduction of a nitro group on the coumarin ring improved the antibacterial activity against P. vulgaris (compounds 57g and 57k, inhibition zone \( >16 \) mm), while the coumarin derivatives without the nitro group showed moderate antifungal activity against C. albicans (compounds 57b, 57e and 57f, inhibition zone \( >12 \) mm). All the tested compounds were inactive against S. aureus, E. coli and K. pneumoniae.

**Antitubercular activity**

The treatment of infections caused by Mycobacterium tuberculosis strains has rapidly become a health problem due to the increasing resistance of bacteria to common antitubercular drugs. Moreover, the multidrug-resistant tuberculosis (MDR-TB) coupled with the increasing overlap of the AIDS and tuberculosis pandemics have brought tuberculosis among the major worldwide health problems. The development of new classes of antitubercular drugs containing a core of 2-amino-1,3,4-thiadiazole moiety is a very challenging task to many scientists.

Antibacterial quinolones became interesting medicines because of the broad antibacterial spectrum on both...
Gram-positive and Gram-negative bacteria. Fluoroquinolone derivatives have been introduced in therapy since 1980s and have multiple recommendations, some of them (eg, gatifloxacin, sparflaxacin and moxifloxacin) being also used in tuberculosis treatment in the cases involving resistance or intolerance to first-line antituberculosis therapy.110,111

A new class of antibacterial fluoroquinolones is represented by sulfonflorquinolones which are hybrid molecules incorporating a sulfonamide moiety and a fluoroquinolone ring. Sulfonflorquinolones are particularly active against Gram-positive bacteria. Thus, Talath and Gadad112 have synthesized several 7-[4-(5-amino-1,3,4-thiadiazol-2-sulfonyl)piperazin-1-yl]fluoroquinolone derivatives such as 58. The unsubstituted cyclopropyl 58a and ethyl 58b derivatives showed moderate activity against M. tuberculosis strain H₃₇Rv at MIC of 10 μg/mL with respect to reference drug, isoniazid. The studied compounds showed antibacterial activity against other Gram-positive bacteria (S. aureus, E. faecalis, Bacillus sp., MIC =1–5 μg/mL) better than the reference fluoroquinolones (ciprofloxacin, norfloxacin, sparflaxacin and gatifloxacin) and almost similar activity as reference drugs against Corynebacterium sp. The best activity was exhibited by the derivatives 58c and 58d, which were active against all the tested Gram-positive bacteria (MIC =1–5 μg/mL). All derivatives exhibited poor activity against Gram-negative bacteria. These results are in agreement with some earlier reports which showed that substitution with aryl/heteroaryl groups at C-7 of fluoroquinolone ring is responsible for distinguishing between antibacterial activity against Gram-positive and Gram-negative bacteria.112–114 The reason might be differences in the structure of the cell wall of Gram-positive bacteria and Gram-negative bacteria. The cell walls of the Gram-positive bacteria contain peptidoglycan and bacterium may or may not be surrounded by a polysaccharide envelope. The cell walls of the Gram-negative bacteria contain lipopolysaccharides. The attack site of anti-cell wall agents is the peptidoglycan layer which is essential for the survival of bacteria. The damage of this layer leads to changes in the bacterial cell wall resulting in death.115 In the case of compounds such as quinolones, which act as antibacterial agents by different mechanism, the structure of the cell wall is still important. The peptidoglycan layer of the Gram-positive bacteria allows the hydrophobic compounds to cross the cell wall and penetrate into the cell, while the lipopolysaccharides of the Gram-negative bacteria repel the hydrophobic compounds. The impermeable cell wall makes the Gram-negative bacteria more resistant to large or intensively hydrophobic molecules.6

New α-[5-(5-amino-1,3,4-thiadiazol-2-yl)-imidazol-2-ythio] acetic acid derivatives such as 59 were in vitro evaluated for antitubercular activity against M. tuberculosis strain H₃₇Rv. Compared to standard drug, rifampicin, the tested compounds exhibited low activity (MIC $6.25 μg/mL, inhibition ≤24%), but they might be considered as good starting compounds for better antitubercular agents.116 In comparison, the dichlorobenzothiophene thiadiazole 44c exhibited very high activity against M. tuberculosis strain H₃₇Rv in BACTEC 12B medium (98% inhibition) at the concentration of 6.25 μg/mL.97

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**Table 1**: Chemical structures of 58 derivatives

| R | R₁ | R₂ | R₃ | R₄ |
|---|---|---|---|---|
| a |   | H | H | H |
| b | C₂H₅ | H | H | H |
| c | NH₂ | F | CH₃ | CH₃ |
| d | H | OCH₃ | H | CH₃ |
Karakus and Rollas\textsuperscript{117} have synthesized new \(N\)-phenyl-\(N\')-[4-(5-alkyl/arylamino-1,3,4-thiadiazol-2-yl)phenyl]thio-ureas 60. Using BACTEC 460 radiometric system (Becton Dickinson, Cockeysville, MD, USA), the in vitro activity against \(M.\) \textit{tuberculosis} strain \(H_37\) \(Rv\) at 6.25 \(\mu\)g/mL has been studied. Compared to standard drug, rifampicin, the best inhibitory activity (67\%) was shown by the derivative 60a having a cyclohexyl group, while the derivative 60b having a \(p\)-chlorophenyl group showed 32\% inhibition against \(M.\) \textit{tuberculosis}. Other derivatives exhibited different degrees of inhibition (16\% for compound 60c, 6\% for compound 60d).

Similarly, 2-amino-5-R-1,3,4-thiadiazole derivatives such as 61 were investigated for antitubercular activity. Among the tested compounds, 2-phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole 61e showed the best inhibitory activity (69\%), while 2-phenylamino-5-phenyl-1,3,4-thiadiazole 61a showed 65\% inhibitory activity against in vitro growing \(M.\) \textit{tuberculosis} \(H_37\) \(Rv\) at a concentration of 6.25 \(\mu\)g/mL. Other studied molecules were classified as high activity compounds (28 derivatives, inhibition activity \(\geq 35\%\)), and this structure may be considered a good scaffold for the development of new antituberculosis agents. The electronic topological method (ETM) and feed-forward neural networks (FFNNs), which were used for the structure–antituberculosis activity relationship study, gave a system of pharmacophores and anti-pharmacophores able to separate the examined compounds in active and inactive compounds. According to these methods, low-activity molecules form a buffer zone consisting of compounds that can include both pharmacophores and anti-pharmacophores.\textsuperscript{42}

The antimycobacterial activity against \(M.\) \textit{tuberculosis} of 1,3,4-thiadiazole derivatives bearing an imidazo[2,1-b]thiazole moiety has been evaluated, the most active compound being the phenylamino derivative 62 (16\% inhibition, MIC > 6.25 \(\mu\)g/mL).\textsuperscript{102} Vasoya et al\textsuperscript{95} have reported the antitubercular activity against \(M.\) \textit{tuberculosis} of some 2-(3'-chloro-5'-phenoxybenzo[b]thiophen-2'-yl)-5-arylamino-1,3,4-thiadiazole derivatives such as 41. In comparison to the antimicrobial screening of the same compounds, the derivatives bearing the electron-donating groups (eg, methyl and methoxy) at C-2 and C-4 of the arylamino group showed higher activity, the most active compound being 2-(3'-chloro-5'-phenoxybenzo[b]thiophen-2'-yl)-5-(p-methoxyphenyl)amino-1,3,4-thiadiazole 41e (91\% inhibition at the concentration of 6.25 \(\mu\)g/mL).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Ar & R & Inhib (\%) \\
\hline
a & \(C_6H_5\) & 65 \\
b & \(C_6H_5\) & 50 \\
c & 4-ClC_6H_4 & 50 \\
d & 4-ClC_6H_4 & 54 \\
e & 4-FC_6H_4 & 69 \\
f & 4-FC_6H_4 & 52 \\
g & 4-C_6H_4N & 53 \\
h & 4-C_6H_4N & 59 \\
\hline
\end{tabular}
\end{table}

New \(N\)-[5-(1-aminoo-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-7-substituted 1,3-benzothiazol-2-amine derivatives such as 63 have been in vitro evaluated for antitubercular activity against \(M.\) \textit{tuberculosis} strain \(H_37\) \(Rv\). The compounds showed promising antitubercular activity in comparison to standard drugs, rifampicin and isoniazid.\textsuperscript{118} Bayrak et al\textsuperscript{108} hybridized antipyrine with 1,3,4-thiadiazole and 1,2,4-triazole rings,
getting the compounds such as 55. Even if the synthesized compounds were completely inactive against Gram-positive microorganisms such as S. aureus, E. faecalis or B. cereus, the studied compounds exhibited good inhibitory activity (inhibition zone of 25 mm at 5 μg/mL) against Mycobacterium smegmatis which is an atypical factor of tuberculosis.

Some thiadiazole derivatives designed as M. tuberculosis inhibitors were tested using BACTEC 460 radiometric system. The antitubercular activity of 2-(alkyl/arylamino)-5-(4-aminophenyl)-1,3,4-thiadiazoles 64 was compared to those of the Schiff bases 65 obtained by the condensation of the starting thiadiazoles with aromatic and heterocyclic aldehydes. Some Schiff bases synthesized from salicylaldehyde and 3-nitrobenzaldehyde and bearing an arylamino group at C-2 of thiadiazole ring were more active than the starting thiadiazoles (eg, compounds 65c-f). All the Schiff bases having an alkylamino group at C-2 of thiadiazole ring were less active than the starting thiadiazoles (compounds 65a-b). 2-Phenylamino-5-[4-(2-hydroxybenzylideneamino)phenyl]-1,3,4-thiadiazole 65c showed the highest inhibition (51%, MIC 6.25 μg/mL), which is in agreement with other studies indicating that the conjugation of hydroxyl ligand with the imine group synergistically enhances the antimycobacterial activity.

Other hybrid molecules incorporating the 1,3,4-thiadiazole ring and benzimidazole (47), benzoxazole (48) and benzothiazole (49) moiety were screened for in vitro antitubercular activity against M. tuberculosis H₃⁷Rv strain using the microplate Alamar blue assay (MABA). Compounds demonstrating at least 90% inhibition in preliminary screening were further evaluated to determine the MIC. All the compounds exhibited inhibitory activity against M. tuberculosis with a rate of inhibition between 53% and 95% at a concentration of 6.25 μg/mL. Out of all heterocyclic derivatives, the benzoxazole derivatives such as 48 have shown the best inhibitory activity (93% for 48a and 95% for 48b), while the benzimidazole derivatives such as 47 have shown the moderate inhibitory activity (77% for 47a and 74% for 47b). 29

| R₁        | Inhib (%) |
|-----------|-----------|
| CH₃       | 29        |
| CH₂CH₃    | 34        |
| CH₃CH₂    | 16        |
| 4-CH₂CH₃  | 22        |
Starting from L-methionine, Tatar et al. synthesized different phenylamino-1,3,4-thiadiazole derivatives. 1,3,4-Thiadiazole benzamide and 1,3,4-thiadiazole thiourea exhibited antimycobacterial activity against M. tuberculosis H₃₇Rv strain with MIC values of 79.50 and 61.77 \( \mu \)M, respectively. Unfortunately, the compounds proved low selectivity with minimum cytotoxic concentration (MCC) values lower than MIC values.

The fungicidal activity of thiadiazole derivatives against F. oxysporum, Gibberella zeae, A. niger and Harpophora oryzae has been previously reported. Some sulfide, sulfoxide and sulfonamide thiadiazoles were also reported for their fungicidal activity against G. zeae, B. cinerea, Sclerotinia sclerotiorum, F. oxysporum or Cytospora mandshurica. In this connection, various derivatives containing 2-amino-1,3,4-thiadiazole system have been prepared. 2-Arylamino-5-glycosyl-1,3,4-thiadiazole derivatives such as 68 were synthesized by Zong et al. and the fungicidal activity against six fungal species: S. sclerotiorum, Phytophthora parasitica, B. cinerea, Rhizoctonia solani, Pyricularia oryzae and Phoma asparagi was in vitro investigated. Most of the 20 studied compounds displayed good fungicidal activities against S. sclerotiorum (12 compounds with inhibition rate of \( \geq 85% \)) and P. oryzae (seven compounds with inhibition rate of \( \geq 80% \)) at a concentration of 50 \( \mu \)g/mL. Further studies against S. sclerotiorum at the concentrations of 50, 20, 10, 5 and 2 \( \mu \)g/mL were used to determine the effective concentration values EC\(_{50}\). About half of the studied compounds displayed good fungicidal activity with EC\(_{50}\) < 3 \( \mu \)g/mL. The SAR studies showed that the compounds with two electron-withdrawing groups at the benzene ring exhibited the best fungicidal activity. Thus, compounds 68b (EC\(_{50}\) = 0.29 \( \mu \)g/mL) and 68j (EC\(_{50}\) = 0.46 \( \mu \)g/mL) are comparable with the commercial fungicide, chlorothalonil. In comparison, the compounds with two electron-donating groups at the benzene ring, such as 68a and 68f, displayed only moderate fungicidal activity. Furthermore, SAR studies revealed the importance of the hydroxyl protecting group in the sugar ring. Thus, the compounds without any hydroxyl group protection in the sugar ring displayed a significant decrease in their fungicidal activity. In addition, the allyl protecting group was more efficient for the fungicidal activity compared to the acetyl protecting group. In general, the allyl derivatives displayed better fungicidal activity against the six fungal species than the acetyl derivatives (eg, 68c and 68h, 68d and 68i and 68e and 68j).

| Inhib (%) | R₁ | R₂ |
|-----------|----|----|
| 0         | 2-HOC₆H₅ | C₆H₅ |
| 0         | 3-NO₂C₆H₄ | C₆H₅ |
| 51        | 2-HOC₆H₅ | C₆H₅ |
| 29        | 3-NO₂C₆H₄ | C₆H₅ |
| 36        | 2-HOC₆H₅ | 4-CH₃C₆H₄ |
| 34        | 3-NO₂C₆H₄ | 4-CH₃C₆H₄ |

Fungicidal and herbicidal activities
The fungicidal activity of thiadiazole derivatives against A. niger and Harpophora oryzae and their potential application as pesticides in agriculture has been previously reported. Some sulfide, sulfoxide and sulfonamide thiadiazoles were also reported for their fungicidal activity against Gibberella zeae, Botrytis cinerea, Sclerotinia sclerotiorum, F. oxysporum or Cytospora mandshurica. In this connection, various derivatives containing 2-amino-1,3,4-thiadiazole system have been prepared. 2-Arylamino-5-glycosyl-1,3,4-thiadiazole derivatives such as 68 were synthesized by Zong et al. and the fungicidal activity against six fungal species: S. sclerotiorum, Phytophthora parasitica, B. cinerea, Rhizoctonia solani, Pyricularia oryzae and Phoma asparagi was in vitro investigated. Most of the 20 studied compounds displayed good fungicidal activities against S. sclerotiorum (12 compounds with inhibition rate of \( \geq 85% \)) and P. oryzae (seven compounds with inhibition rate of \( \geq 80% \)) at a concentration of 50 \( \mu \)g/mL. Further studies against S. sclerotiorum at the concentrations of 50, 20, 10, 5 and 2 \( \mu \)g/mL were used to determine the effective concentration values EC\(_{50}\). About half of the studied compounds displayed good fungicidal activity with EC\(_{50}\) < 3 \( \mu \)g/mL. The SAR studies showed that the compounds with two electron-withdrawing groups at the benzene ring exhibited the best fungicidal activity. Thus, compounds 68b (EC\(_{50}\) = 0.29 \( \mu \)g/mL) and 68j (EC\(_{50}\) = 0.46 \( \mu \)g/mL) are comparable with the commercial fungicide, chlorothalonil. In comparison, the compounds with two electron-donating groups at the benzene ring, such as 68a and 68f, displayed only moderate fungicidal activity. Furthermore, SAR studies revealed the importance of the hydroxyl protecting group in the sugar ring. Thus, the compounds without any hydroxyl group protection in the sugar ring displayed a significant decrease in their fungicidal activity. In addition, the allyl protecting group was more efficient for the fungicidal activity compared to the acetyl protecting group. In general, the allyl derivatives displayed better fungicidal activity against the six fungal species than the acetyl derivatives (eg, 68c and 68h, 68d and 68i and 68e and 68j).

| EC\(_{50}\) | R₁ | R₂ |
|----------|----|----|
| 7.11     | COCH₃ | 2.4-(CH₃)₂C₆H₃ |
| 0.29     | COCH₃ | 3.4-ClC₆H₅ |
| 3.94     | COCH₃ | 2.5-ClC₆H₅ |
| 22.53    | COCH₃ | 1-Naphthyl |
| 1.50     | COCH₃ | 4-Cl-3-CF₃C₆H₃ |
| 9.92     | allyl | 2.4-(CH₃)₂C₆H₃ |
| 1.50     | allyl | 3.4-ClC₆H₅ |
| 1.62     | allyl | 2.5-ClC₆H₅ |
| 2.0     | allyl | 1-Naphthyl |
| 0.46     | allyl | 4-Cl-3-CF₃C₆H₃ |
Yadav et al\textsuperscript{125} have synthesized 2-amino-5-substituted-1,3,4-thiadiazoles 69, and the in vitro fungicidal activity against \textit{P. oryzae} and \textit{R. solani} at 500 ppm was investigated. Fungicidal screening data indicated a moderate activity compared to the reference fungicide, carbenzadim, the tested compounds being more active against \textit{P. oryzae} than \textit{R. solani}. The nature and the position of the substituents of the phenyl ring significantly changed the biological activity. Among the tested compounds, the chlorosubstituted derivatives 69a, 69b and 69d have shown greater activity (\textit{P. oryzae} inhibition rate between 32\% and 42\%) than the methylated ones (eg, 69c, \textit{P. oryzae} inhibition rate of 16\%), the \textit{para} chlorinated compound 69a being the most active. Methylation of the \textit{p}-chloroderivative decreased the activity on both fungi.

Chu et al\textsuperscript{126} have synthesized several \textit{α}-(5-arylamino-1,3,4-thiadiazol-2-thio)-\textit{α}-(1\textit{H}-1,2,4-triazol-1-yl)aceto phenone derivatives such as 70 which exhibited, at a concentration of 50 ppm, fungicidal activity against some pathogenic fungi of plants such as leaf rust of barley, leaf spot of beet, early blight of tomato, gray mold of cucumber and sclerotium blight of colza with an inhibition rate between 5\% and 66.6\%. Chemical modification of these structures might give good fungicidal agents for agriculture.

In addition, pyridazinone-substituted 1,3,4-thiadiazoles 71 synthesized by Zou et al\textsuperscript{127,128} were tested for in vivo antifungal activity against wheat leaf rust, \textit{Puccinia recondita}, at a concentration of 0.001 M solution in water/dimethylformamide. Some derivatives showed high inhibitory activity (80\%–90\%) compared to the commercial fungicide, triadimefon (100\% inhibition). A quantitative SAR (QSAR) study showed that the increase in the hydrophobicity of \( R_2 \) leads to an increased fungicidal activity (\( m-\text{CF}_3 > o-\text{F} > \text{H} \)). Concerning the substituent \( R_1 \), the best and consistent results were obtained in the cases of \textit{ortho} substituents with inductive electron-donating properties. The preferred substituent for \( R_1 \) was the 2,4-dimethyl group, as can be seen with compounds 71d and 71e.

![Diagram](image1)

**Conclusion**

Thiadiazole ring is present in compounds with various biological activities. Among the different isomers of thiadiazole, 1,3,4-thiadiazole derivatives are most studied due to broad spectrum of pharmacological activities. Although only a few pharmacological effects exhibited by 1,3,4-thiadiazole derivatives are currently clinically used (eg, antibacterial activity and carbonic anhydrase inhibiting activity), the substitution at thiadiazole ring is a challenging approach to obtain agents with improved potency and less toxicity.

The cytostatic properties of 2-amino-1,3,4-thiadiazole and the anti-trypanosomal properties of megazol are evidence of the biological potential of the 2-amino-1,3,4-thiadiazole moiety. Literature surveys report the antimicrobial activity of substituted 2-amino-1,3,4-thiadiazoles, making 2-amino-1,3,4-thiadiazole a unique template with significant utility in medicinal chemistry. Many 2-amino-1,3,4-thiadiazole derivatives can be considered as lead compounds for drug development. The SAR studies revealed that the antimicrobial activity is dependent on the nature of the substituents at the thiadiazole nucleus.

Although the antibacterial, antifungal and antitubercular properties are the main antimicrobial activities studied, other antimicrobial activities exhibited by 2-amino-1,3,4-thiadiazole derivatives are also explored. Many derivatives have shown good antimicrobial activities with good in vitro
experimental results but deficient in vivo experimental data, suggesting that the mechanism of action is not clear and further studies are needed.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Wermuth CG, Ciapetti P, Giethlen B, Bazzini P. Bioisosterism. Comprehensive Medicinal Chemistry. Vol. 2. Taylor JB, Triggle DJ, editors. Amsterdam: Elsevier; 2007:649–711.
2. Holla BS, Poorjary KN, Rao BS, Shivananda MK. New bis-aminomercaptopiazolones and bis-triazolothiadiazoles as possible anticancer agents. Eur J Med Chem. 2002;37:511–517.
3. Yousif E, Majeed A, Al-Sammarrae K, Salih N, Salimon J, Abdullah B. Metal complexes of Schiff base: preparation, characterization and antibacterial activity. Arabian J Chem. 2013;6(52). doi: 10.1016/j.arabjc.2013.06.006.
4. Li Y, Geng J, Liu Y, Yu S, Zhao G. Thiazole – a new chemo therapeutic agent. J Am Chem Soc. 1955;77:6713–6714.
5. Wermuth CG, Aldous D, Raboisson P, Rogan D. The Practice of Medicinal Chemistry. 4th ed. London: Academic Press, Elsevier; 2015;196.
6. Hu Y, Li CY, Wang XM, Yang YH, Zhu HL. 1,3,4-Thiadiazole: synthesis, reactions and applications in medicinal, agricultural, and materials chemistry. Rev Chem. 2014;11(10):5722–5760.
7. Brown N. Bioisosteres in Medicinal Chemistry. Weinheim, Germany: Wiley-VCH Verlag and Co., KGaA; 2012.
8. Tripathy R, Ghose A, Singh J, et al. 1,2,3-Thiadiazole substituted pyrazolones as potent KDR/VEGFR-2 kinase inhibitors. Bioorg Med Chem Lett. 2007;17(6):1793–1798.
9. Wermuth CG. Are pyridazines privileged structures? Drug Disc. 2011;2:935–941.
10. Biziare K, Worms P, Kan JP, Mandel P, Garattini S, Roncucci R. Minaprine, a new drug with antidepressant properties. Drugs Exp Clin Res. 1985;11(12):831–840.
11. Ueno S, Bracanontes J, Zorunski C, Weiss DS, Steinbach JH. Bicuculline and gabazine are allosteric inhibitors of channel opening of the GABA A receptor. J Neurosci. 1997;17(2):625–634.
12. Senff-Ribeiro A, Echevarria A, Silva EF, Franco CRC, Veiga SS, Oliveira MBM. Cytotoxic effect of a new 1,3,4-thiadiazolium mesoionic compound (MI-D) on cell lines of human melanoma. J Pharm Sci. 2002;91(2):297–304.
13. Fosbinder RJ, Walter LA. Sulfanilamido derivatives of heterocyclic amines. J Am Chem Soc. 1939;61(8):2032–2033.
14. Lott WA, Bergem FH. 2-(p-Aminobenzenesulfonamido)-thiazole: a new chemo therapeutic agent. J Am Chem Soc. 1939;61:3593–3594.
15. Tapp O. Heterocyclic derivatives of sulfanilamide. Recl Trav Chim. 1943;62:207–209.
16. Masaki O, Mifune A, Higashijima T, Nagato S. 1,3,4-Thiadiazole derivatives. Several reactions of aminothiadiazoles. J Pharm Soc Jpn. 1951;71:481–484.
17. Frisk AR. Sulfanilamide derivatives, chemotherapeutic evaluation of N-,sulfonated substituents. Acta Med Scand Suppl. 1943:142:1.
18. Beale JM, Block JH. Wilson and Gisvold’s Textbook of Organic Medicinal and Pharmaceutical Chemistry. 12th ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer; 2011:238.
19. Roblin RO, Clapp JW. Carbonic anhydrase inhibitor. J Am Chem Soc. 1950;72:4890.
20. Clapp JW, Roblin RO. US Patent 2554816. 1951; Chem Abstr. 1952;46:3087.
21. Seldin D, Giebisch G. Diuretic Agents: Clinical Physiology and Pharmacology. San Diego: Academic Press; 1997:24.
22. Lemke TL, Williams DA, Roche VF, Zito SW. Foye’s Principles of Medicinal Chemistry. 7th ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer; 2013:735.
23. Medindex [homepage on the Internet]. Available from: http://medindex.nlm.nih.gov/index.php?term=UMLS-CSP-HL7-ICD9CM-NCI-NDFRT-RXNORM, METHAZOLAMIDE.shtml. Accessed March 7, 2017.
24. Olsen JJ, Sloboda A, Troy WP, et al. The carcinostatic activity of some 2-amino-1,3,4-thiadiazoles. J Am Chem Soc. 1955;77:6713–6714.
25. Berkelhammer G, Asato G. 2-Amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole: a new antimicrobial agent. Science. 1968;162(3858):1146.
26. Marie-Daragon A, Rouillard MC, Boutelle B, Bisser S, de Albuquerque C. An efficacy trial on Trypanosoma brucei of molecules permeating the blood-brain barrier and of megazol. Bull Soc Pathol Exot. 1994;87(5):347–352.
27. Boutelle B, Marie-Daragon A, Chauviere G, de Albuquerque C, Enanga B. Effect of megazol on Trypanosoma brucei acute and subacute infections in Swiss mice. Acta Trop. 1995;60(2):73–80.
28. Nisslany F, Brugier S, Mouriès MA, Le Curieux F, Marzin D. In vitro and in vivo chromosomal aberrations induced by megazol. Mutat Res. 2004;560(2):147–158.
29. Enanga B, Ariyanayagam MR, Stewart ML, Barrett MP. Activity of megazol, a trypanocidal nitroimidazole, is associated with DNA damage. Antimicrob Agents Chemother. 2003;47(10):3368–3370.
30. de Silva Carvalho A, Salomao K, de Castro SL, et al. Megazol and its biosiostrere 4-H-1,2,4-triazole: comparing the trypanocidal, cytotoxic and genotoxic activities and their in vitro and in silico interactions with the Trypanosoma brucei nitroreductase enzyme. Mem Inst Oswaldo Cruz. 2014;109(3):315–322.
31. Chauviere G, Boutelle B, Enanga B, et al. Synthesis and biological activity of nitro heterocyles analogous of megazol, a trypanocidal lead. J Med Chem. 2003;46(3):427–440.
32. Nishida M, Matsubara T, Murakawa T, Mine Y, Yokota Y, Kuwahara S. In vitro and in vivo evaluation of cefazolin, a new cephalosporin C derivative. Antimicrob Agents Chemother (Bethesda). 1969;9:236–243.
33. Nishida M, Matsubara T, Murakawa T, Mine Y, Yokota Y, Cefazolin, a new semisynthetic cephalosporin antibiotic. II. In vitro and in vivo antimicrobial activity. J Antimicrob (Tokyo). 1970;23(3):137–148.
34. Kusaba T. Safety and efficacy of cefazolin sodium in the management of bacterial infection and in surgical prophylaxis. Clin Med Insights Ther. 2009;1:1607–1615.
35. Saginur R, Croteau D, Bergeron MG. Comparative efficacy of teicoplanin and cefazolin for cardiac operation prophylaxis in 3027 patients. The ESPRIT Group. J Thorac Cardiovasc Surg. 2000;120(6):1120–1130.
36. Maki DG, Bohn MJ, Stolz SM, Kornecke GM, Acher CW, Myrowitz PD. Comparative study of cefazolin, cefamandole and vancomycin for surgical prophylaxis in cardiac and vascular operations. A double-blind randomized trial. J Thorac Cardiovasc Surg. 1992;104(5):1423–1434.
37. Finkelstein R, Rabino G, Mashiah T, et al. Vancomycin versus cefazolin prophylaxis for cardiac surgery in the setting of a high prevalence of methicillin-resistant staphylococcal infections. J Thorac Cardiovasc Surg. 2002;123(2):326–332.
38. Bolon MK, Morlote M, Weber SG, Koplan B, Carmeli Y, Wright SB. Glycopeptides are no more effective than beta-lactam agents for prevention of surgical infection after cardiac surgery: a meta-analysis. Clin Infect Dis. 2004;38(10):1357–1363.
39. Amir M, Kumar A, Ali I, Khan SA. Synthesis of pharmaceutically important 1,3,4-thiadiazole and imidazoline derivatives as antimicrobials. Indian J Chem. 2009;48B:1288–1293.
40. Desai K, Baxi AJ. Studies on 2-azetidinone: part VI synthesis and antimicrobial activity of 5-(2′,4′-dichlorophenoxyethyl)-2-(4′-aryl-3′-chlo-ro-2′-azetidinone-1′-yl)1,3,4-thiadiazole. Indian J Pharm Sci. 1992;54:183–188.
41. Salimon J, Salih N, Hameed A, Ibraheem H, Yousef E. Synthesis and antibacterial activity of some new 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives. J Appl Sci Res. 2010;6(7):866–870.
42. Oruc EE, Rollas S, Kandemirli F, Shvets N, Dimoglo A. 1,3,4-Thiadiazole derivatives. Synthesis, structure elucidation and structure-antituberculosis activity relationship investigation. J Med Chem. 2004; 47(27):6560–6567.
43. Bonina L, Iannello D, Merendino R, Arena A, Mastroeni P. Tumor-dependent resistance of rat peritoneal macrophages to herpes simplex virus. Drugs Exp Clin Res. 1983;9:577–583.
44. Schenone S, Brullo C, Bruno O, et al. New 1,3,4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities. Bioorg Med Chem. 2006;14(6):1698–1705.
45. Clerici F, Pocar D, Guido M, Loche A, Perlini V, Brufani M. Synthesis of 2-amino-5-sulfonyl-1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. J Med Chem. 2001;44(6):931–936.
46. Turner S, Myers M, Gadie B, et al. Antihypertensive thiadiazoles. 2. Rifampicin-dependent resistance of rat peritoneal macrophages to herpes simplex virus. Drugs Exp Clin Res. 1983;9:577–583.
47. Labanauskas L, Kalcas V, Udrenaitė E, Gaidelis P, Brukstas A, Duruksi A. Synthesis of 3-(3,4-dimethoxyphenyl)-1H,1,2,4-triazole-5-thiol and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole derivatives exhibiting anti-inflammatory activity. Pharmazie. 2001;56(8):617–619.
48. Pattan SR, Desai NS, Bukitgar AA, Wakale VA. Synthesis and antimicrobial evaluation of some 1,3,4-thiadiazole derivatives. Indian J Pharm Ed Res. 2008;4(2):314–318.
49. Mazzzone G, Pignatello R, Mazzone S, et al. Synthesis and local anesthetic activity of alkylaminoarylcarbonyl derivatives of 2-amino-1,3,4-thiadiazole. Farmaco. 1993;48(9):1207–1224.
50. Siddiqui A, Dai C, Mansoor U, Yang L, Vitharana L, Angeles A. Spirocondensed 1,3,4-thiadiazole derivatives for inhibiting KSP kinesin activity. J Biochem. 2009;105:8228.
51. Basso A, Liu M, Dai C, et al. SCH 2047069, a novel oral kinesin spindle protein inhibitor, shows single-agent antitumor activity and enhances the efficacy of chemotherapeutics. Mol Cancer Ther. 2010;9(11):2993–3002.
52. Taha SS, Ahmad AA, Mavlood SI, Ali NO. Synthesis of some series of 2-aryl-5-guanidino-1,3,4-thiadiazoles. J Med Chem. 2008;45(11):5006–5011.
53. Liu G, Cogan DA, Ellman JA. Catalytic asymmetric synthesis of tert-butanesulfonamide. Application to the asymmetric synthesis of amines. J Am Chem Soc. 1997;119(41):9913–9914.
54. Kadi AA, Al-Abdullah ES, Shehata IA, Habib EE, Ibrahim TM, El-Emam AA. Synthesis, antimicrobial and anti-inflammatory activities of novel 5-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. Eur J Med Chem. 2007;42(2):235–242.
55. Shah H, Vashi S, Mehta S. Synthesis of 2,5-disubstituted-1,3,4-oxadiazole, 1,5-disubstituted-2-mercaptop-1,3,4-triazole and 2,5-disubstituted-1,3,4-thiadiazole as potential therapeutic agents. Indian J Chem. 1996;35B:111–115.
56. Nargund GVL, Reddy NRG, Hariprasad N. Synthesis and antibacterial activity of a series of 1H-2-mercapto-5-[4-(acetamidophenoxy)methyl]-1,3,4-thiazoles, thiadiazoles and 2-[4-(acetamidophenoxy carbonyl)]3,4,5-trisubstituted pyrazoles. Indian J Chem. 1996;35B:499–502.
57. Sah P, Bidawat P, Seth M, Gharu CP. Synthesis of formazans from Mannich base of 5-(4-chlorophenylamino)-2-mercaptop-1,3,4-thiadiazole as antimicrobial agents. Arzhan Chem. 2014;7:181–187.
58. Dogan HN, Duran A, Rollas S, Sener G, Uysal MK, Gulen D. Synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. Bioorg Med Chem. 2002;10(9):2893–2898.
59. Farshori NN, Banday MR, Ahmad A, Khan AU, Rauf A. Synthesis, characterization and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and thiadiazoles. Bioorg Med Chem Lett. 2010(6):1933–1938.
60. Barbouz M, Cimpeanu M, Guran C, Supuran CT. 1,3,4-Thiadiazole derivatives. Part 9. Synthesis and biological activity of metal complexes of 5-(2-aminoethyl)-2-aminoo-1,3,4-thiadiazole. Met Based Drugs. 1996;3(5):227–232.
61. Obaleye JA, Adejiyi JJ, Adebayo MA. Synthesis and biological activities on metal complexes of 2,5-diamoio-1,3,4-thiadiazole derived from semicarbazide hydrochloride. Molecules. 2011;16(7):5861–5874.
62. Ashutosh B, Ankur J, Kumar NR, et al. Synthesis, characterization and antimicrobial activity of azol substituted derivatives. Int J Pharm Sci Res. 2009;1(3):207–210.
63. Rezki N, Al-Yahewi AM, Bardawlew SK, Al-Blewi FF, Aouad MR. Synthesis of novel 2,5-disubstituted-1,3,4-thiadiazoles clubbed 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole and/or Schiff base as potent antimicrobial and antiproliferative agents. Molecules. 2015; 20(9):16048–16067.
64. He Y-W, Cao L-H, Zhang J-B, Wang D-Z, Aisa HA. Synthesis and biochemical characterization and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and thiadiazoles. Molecules. 2011;16(7):5861–5874.
65. Hussain S, Sharma J, Amir M. Synthesis and Antimicrobial activities of 1,2,4-triazole and 1,3,4-thiadiazole derivatives of 5-amino-2-hydroxybenzoic acid. E-J Chem. 2008;5(4):963–968.
66. Kucukguzel SG, Kucukguzel I, Tatari E, et al. Synthesis of some novel heterocyclic compounds derived from difrunil hydrazide as potential anti-infective and anti-inflammatory agents. Eur J Med Chem. 2007;42(7):893–901.
82. Bektas H, Demirbas A, Demirbas N, Bayrak H, Karagolu SA. Synthesis and antimicrobial activities of some new heterocyclic compounds containing 1,2,4-triazol-3-one and 1,3,4-thiadiazole moieties. Turk J Chem. 2010;34:517–527.

83. Demirbas A, Sahin D, Demirbas N, Karagolu SA. Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. Eur J Med Chem. 2009;44(7):2896–2903.

84. Bekht AA, Ashour HMA, Ghany YSA, Bekht AEA, Baraka A. Synthesis and biological evaluation of some thiazolyl and thiazolyl derivatives of 1H-pyrazole as anti-inflammatory antimicrobial agents. Eur J Med Chem. 2008;43(3):456–463.

85. Zamani K, Faghhi K, Tofighi T, Shariatladeh MR. Synthesis and antimicrobial activity of some pyridyl and naphthyl substituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives. Turk J Chem. 2004;28:95–100.

86. Zamani K, Faghhi K, Mehranlou MS. Synthesis of some new 2,5-disubstituted 1,3,4-thiadiazoles containing isocyclic pyridyl as potent antimicrobial agents. Pol J Pharmacol. 2003;55(6):1111–1117.

87. Szulczyk D, Tomaszewski P, Joziwak M, et al. Synthesis and biological activity of ethyl 2-(2-pyridylidacetate) derivatives containing thio-, 1,2,4-triazole, thiazolide and oxadiazole moieties. Molecules. 2011;16(2):409.

88. Patil RL, Mandhare NP, Bondge PS, Munde BS, Mane AR. Synthesis and antimicrobial activity of new pyrimidine incorporated 1,3,4-thiadiazoles. Indian J Heterocycl Chem. 2003;12:245–248.

89. Andrews B, Komathi K, Mohan S. Synthesis and comparing the antibacterial activities of pyrimidine derivatives. J Chem Soc. 2017;129(3):335–341.

90. Klip NT, Capan G, Gunsoy A, Uzun M, Satana D. Synthesis, structure and antifungal evaluation of some novel 1,2,4-triazolymercaptocacetyl thiosemicarbazide and 1,2,4-triazolymercaptomethyl-1,3,4-thiadiazole analogs. J Enzyme Inhib Med Biochem. 2010;25(1):126–131.

91. Onkol T, Dogruer DS, Uzun L, Adak S, Ozkan S, Sahin MF. Synthesis and antimicrobial activity of new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. J Enzyme Inhib Med Biochem. 2008;23(2):277–284.

92. Tehranian S, Akbarzadeh T, Fazelri MR, Jamaliar H, Shafiee A. Synthesis and antibacterial activity of 1-[1,2,4-triazol-3-yl] and 1-[1,3,4-thiazol-2-yl]-3-methylthio-6,7-dihydro-benzo[thiophen-4,5]thiones. Bioorg Med Chem Lett. 2005;15(4):1023–1025.

93. Siddiqui N, Alam MS. 5-((1H-indol-3-ylmethyl)-(N-(substituted phenyl)-1,3,4-thiadiazole-2-amine derivatives: synthesis and biological screening. Biosci Biotechnol Res Asia. 2009;6(1):261–264.

94. Siddiqui N, Ahuja P, Ahsan W, Pandey SN, Alam MS. Thiadiazoles: progress report on biological activities. J Chem Pharm Res. 2009;1(1):19–30.

95. Vasoya SL, Paghdar DJ, Chovatta PT, Joshi HS. Synthesis of some new thiosemicarbazide and 1,3,4-thiadiazole heterocycles bearing benz[b]thiophene nucleus as a potent antitubercular and antimicrobial agents. J Sci I R Iran. 2005;16(1):33–36.

96. Aly AA, El-Sayed R. Synthesis and biological activity of new 1,3,4-thiadiazole derivatives. Chem Pap. 2006;60(1):56–60.

97. Thaker KM, Joshi HS. Synthesis and pharmacological evaluation of 2-(3′,5′-dichlorobenzothiophen-2-yl)-5-arylamino-1,3,4-thiadiazoles. Indian J Chem. 2005;44B:410–412.

98. Ahmad T, Singh AK, Jaiswal N, Singh D. Synthesis and pharmacological activity of 1,3,4-thiadiazole derivatives: a review. Int J Pharm. 2012;3(3):70–82.

99. Hegde VS, Kolavi GD, Lamani RS, Khazi IAM. Dimethylthioimido-carbonates-mediated heterocyclizations: synthesis of imidazolines and benzothiazoles as potent antitubercular agents. Phosphorus Sulfur Silicon. 2007;182(4):911–920.

100. Youssif EM, Hameed A, Kamil A, Farina Y, Asaad N, Graisa A. Synthesis of new polymers derived from poly(vinyl chloride) and study their biological evaluation. Aust J Basic Appl Sci. 2009;3(3):1786–1794.

101. Patel D, Singh A. Synthesis, characterization and antimicrobial activity of metal chelates of [5-(4-chlorophenyl)(1,3,4)thiadiazol-2-ylamino-methylene]-8-hydroxy quinoline. E-J Chem. 2009;6(4):1017–1022.
123. Asif M. A review on pharmacological potentials of various substituted thiadiazole analogs. Int J Cur Res Appl Chem Chem Eng. 2016;2(2):1–26.

124. Zong G, Zhao H, Jiang R, et al. Design, synthesis and bioactivity of novel glycosylthiadiazole derivatives. Molecules. 2014;19(6):7832–7849.

125. Yadav LS, Zaidi MGH, Singh BN. Synthesis and fungicidal evaluation of some 1,3,4-thiadiazoles against P. oryzae and R. solani. Asian J Chem. 2003;15(3):1805–1807.

126. Chu CH, Hui XP, Xu PF, Zhang ZY, Li ZC, Liao RA, Synthesis and antifungal activities of ω-(5-arylamino-1,3,4-thiadiazol-2-thio)-ω-(1H-1,2,4-triazol-1-yl)acetophenones. Indian J Chem. 2002;41B: 2436–2438.

127. Zou XJ, Lai LH, Jin GY, Zhang ZX. Synthesis, fungicidal activity, and 3D-QSAR of pyridazinone-substituted 1,3,4-oxadiazoles and 1,3,4-thiadiazoles. J Agric Food Chem. 2002;50(13):3757–3760.

128. Zou XJ, Jin GY, Zhang ZX. Synthesis, fungicidal activity, and QSAR of pyridazinonethiadiazoles. J Agric Food Chem. 2002;50(6): 1451–1454.