

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

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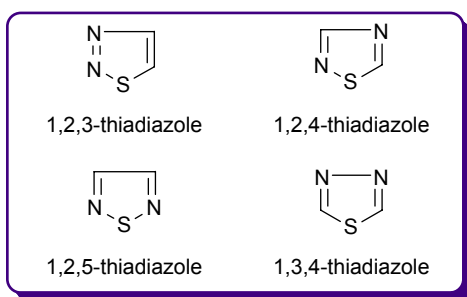


Figure 1 Natural isomers of the thiadiazole ring.

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.³

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 $-\text{CH}=\text{CH}-$ by $-\text{S}-$ (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

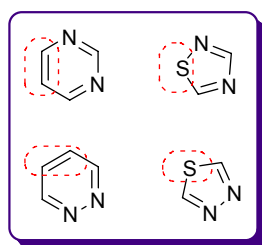


Figure 2 Isosterism between the six-membered diazaheterocycles and thiadiazole derivatives.

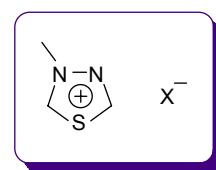
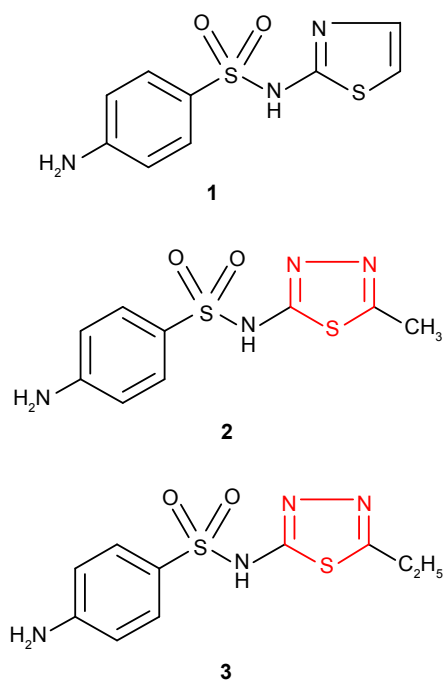


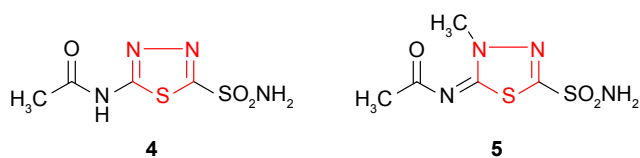
Figure 3 Chemical structure of the mesoionic salt derivatives formed by 1,3,4-thiadiazole compounds.

p and π 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).^{4,12}

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.



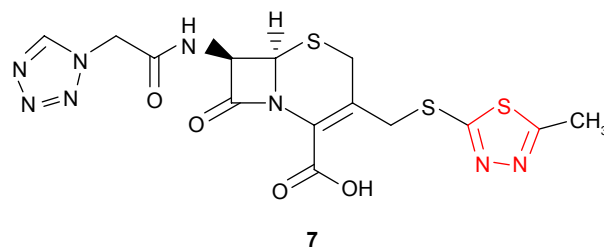
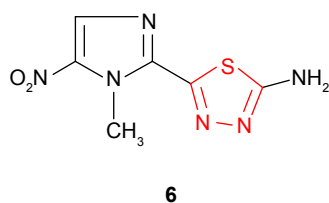
The synthesis of “acetazolamide” **4** (5-acetylamino-1,3,4-thiadiazol-2-sulfonamide) by Roblin and Clapp¹⁹ (Lederle Laboratories, Pearl River, NY, USA) as carbonic anhydrase inhibitor reoriented the researchers to sulfonamides bearing 1,3,4-thiadiazole ring. Acetazolamide was marketed as Diamox (Lederle 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-1*H*-2-imidazolyl]-1,3,4-thiadiazole, CL 64855),²⁵ 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 “cefazolin” **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-thiadiazole 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.⁵⁴ Some of the derivatives included in this 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.⁵⁵

Antibacterial and antifungal activities

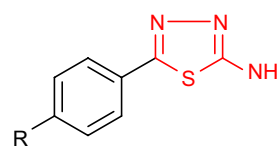
The antimicrobial chemotherapy is the way to combat infections through the pharmacological effects of the drugs used. Antimicrobial agents have specific toxic action on pathogenic organisms. Since the introduction of the first antibiotic (penicillin, 1942) into medical practice, there has been an ongoing race between scientists and pathogenic bacteria.⁵⁶ In the struggle for existence, the microorganisms constantly adapt by selecting higher invasive and more resistant strains. Despite the large number of antibiotics and chemotherapeutics 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.^{55,57} 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.^{56,58–60}

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.⁶¹

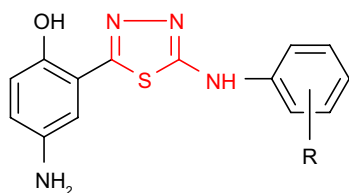
Upadhyay and Mishra⁶² synthesized the 5-(4-substituted phenyl)-1,3,4-thiadiazol-2-amine derivatives **8** and performed in vitro antibacterial activity against *S. aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger* and *Candida albicans* by disk diffusion technique. Fluorinated and chlorinated compounds **8a** and **8b** showed good inhibitory effects (inhibition between 81% and 91%) with minimum inhibitory concentration (MIC) values of 20–28 µg/mL (controlled to ciprofloxacin, MIC = 18–20 µg/mL) for *S. aureus* and *B. subtilis*. In addition, halogenated compounds **8a–c** and hydroxyl derivative **8d** showed moderate inhibitory effects (inhibition between 58% and 79%) with MIC values of 24–40 µg/mL (controlled to ciprofloxacin, MIC = 20–24 µg/mL) for *E. coli* and *P. aeruginosa*. Significant antifungal activity against *A. niger* and *C. albicans* was exhibited by derivatives **8d** and **8e** bearing oxygenated substituents at phenyl ring (inhibition between 58% and 66% and MIC = 32–42 µg/mL compared to fluconazole, MIC = 24–26 µg/mL). It appears that the halogen attached to the phenyl-1,3,4-thiadiazol moieties increases the antibacterial activity with preference against Gram-positive bacteria, while the oxygenated substituents impart antifungal activity.

A new series of 4-amino-2-{5-[(4-substituted phenyl) amino]-1,3,4-thiadiazol-2-yl}phenols **9** was synthesized, and the compounds were in vitro evaluated for their antimicrobial activity. Among the series **9**, the chlorinated and fluorinated derivatives **9d–f** exhibited good antibacterial activity against *S. aureus* and *E. coli* strains and antifungal activity against *A. niger* with MIC values of 25 µg/mL.⁶³



R = F (a); Cl (b); Br (c);
OH (d); OCH₃ (e)

8

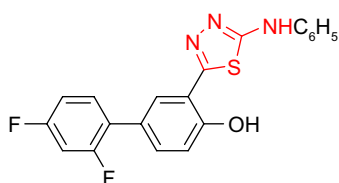


R = 4-CH₃ (a); 4-CH₃O (b); 2,6-(CH₃)₂ (c);
4-Cl (d); 3-Cl-4-F (e); 4-F (f); 4-Br (g)

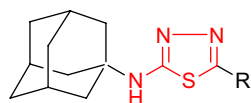
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Other fluorinated compounds incorporating 1,3,4-thiadiazole ring and the diflunisal (a nonsteroidal anti-inflammatory drug) structure were tested for antimicrobial activity using microwell dilution assay and MIC agar dilution assay. 5-(2',4'-Difluoro-4-hydroxybiphenyl-5-yl)-phenylamino-1,3,4-thiadiazole **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.⁶⁴

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*.



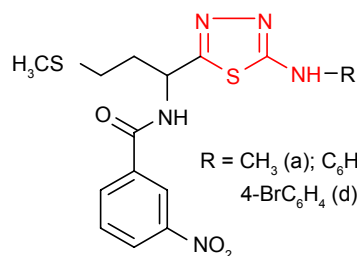
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R = C₆H₅ (a); 4-FC₆H₄ (b); 4-ClC₆H₄ (c);
4-BrC₆H₄ (d); 4-NO₂C₆H₄ (e); 1-adamantyl (f)

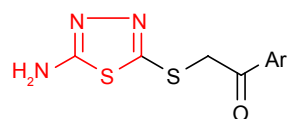
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The in vitro antimicrobial activity of some new 1,3,4-thiadiazole derivatives such as **12** having a D,L-methionine moiety has been evaluated against several bacterial strains by Pintilie et al.⁶⁶ From the findings, the authors revealed that 1,3,4-thiadiazole 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. Even though all the tested compounds showed moderate antibacterial activity against Gram-positive and Gram-negative bacterial strains and moderate to good antifungal activity against *C. albicans*, the findings revealed that the antimicrobial activity was improved by the introduction of a 1-arylethanone moiety 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



12

R = CH₃ (a); C₆H₅ (b); 4-CH₃C₆H₄ (c);
4-BrC₆H₄ (d); H₂C=CHCH₂ (e)

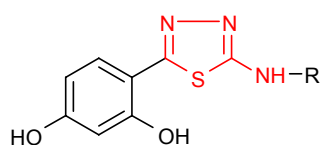


Ar = naphthyl; 4-ClC₆H₄; C₆H₅

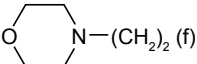
13

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 non-albicans* 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

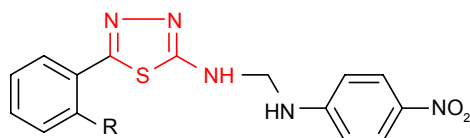
derivative **14f** (MIC =34.4 µg/mL) being the most active compounds. The aminoderivatives substituted with the methyl, phenyl, halogenophenyl, ethoxyphenyl and morpholinoalkyl groups showed higher antifungal activity against *C. albicans* strains. For some of them, such as phenylamino derivative **14b** (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.⁶⁹



R = CH₃ (a); C₆H₅ (b); 2-ClC₆H₄ (c); 2,4-(Cl)₂C₆H₃ (d);

4-C₂H₅OC₆H₄ (e);  (f)

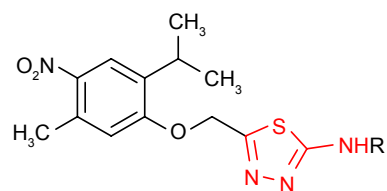
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R = H, OH

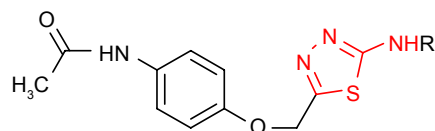
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Some nitrophenoxymethyl-1,3,4-thiadiazole derivatives such as **16** synthesized by Shah et al⁷⁰ were evaluated for antimicrobial activity. The introduction of the nitro group significantly increased the antibacterial activity against *S. aureus*, the studied compounds exhibiting comparable activity to standard drugs, chloramphenicol and ampicillin. In comparison, the acetamidophenoxymethyl-1,3,4-thiadiazole derivatives **17** exhibited moderate antibacterial activities against *B. subtilis*. The introduction of *p*-tolyl substituent (compound **17b**) at the amine group of 1,3,4-thiadiazole ring significantly increased the activity.⁷¹



R = 2,6-(CH₃)₂C₆H₃; 4-CH₃OC₆H₄; 3-CH₃C₆H₄

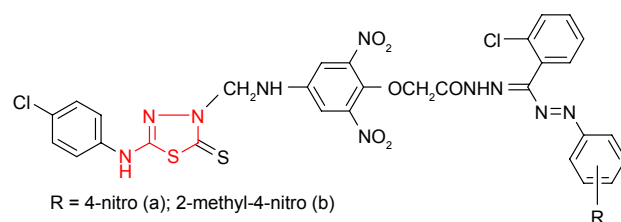
16



R = C₆H₅ (a); 4-CH₃C₆H₄ (b); 4-CH₃OC₆H₄ (c)

17

Having 5-(4-chlorophenylamino)-2-mercapto-1,3,4-thiadiazole as starting material, Sah et al⁷² 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*.



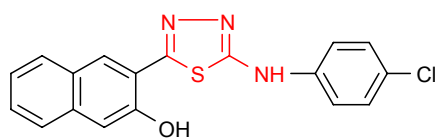
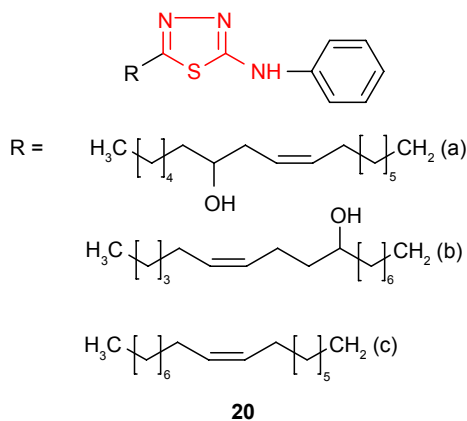
R = 4-nitro (a); 2-methyl-4-nitro (b)

18

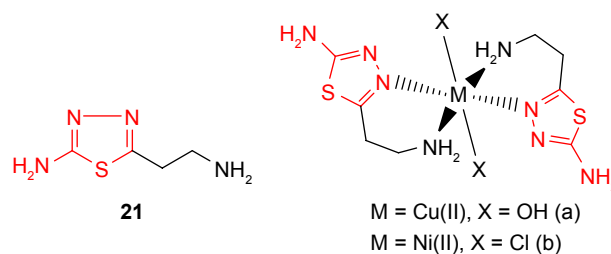
Dogan et al⁷³ have examined the effect of various substitutions at the amine group of 2-amino-5-(3-hydroxy-2-naphthyl)-1,3,4-thiadiazole on antimicrobial activity against different bacterial and fungal strains. Among the series, the *p*-chlorophenyl derivative **19** was marginally active against *S. 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 marneffeii* 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

**19****20**

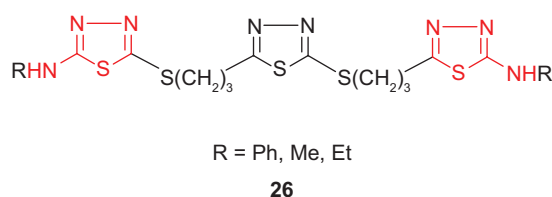
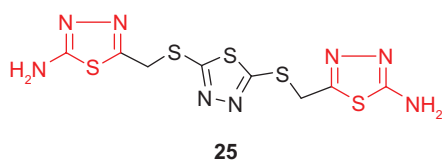
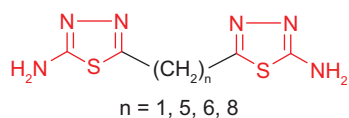
more effective against *Aspergillus flavus*. Obaleye et al⁷⁶ have synthesized 2,5-diamino-1,3,4-thiadiazole **23** as a bioactive ligand and its metal complexes with biologically important ions, such as Co(II), Ni(II) and Cu(II). The ligand behaved as tridentate neutral ligand which coordinated to the metal ions via sulfur and nitrogen of the amines giving solid chelates with general formulae $[(\text{ML}_2)\text{Cl}_2]$ 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* 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.

**21****22****23**

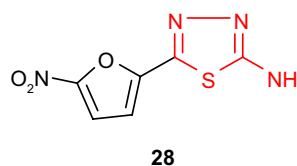
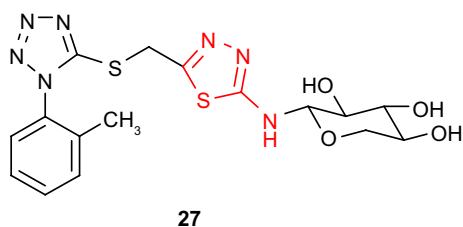
2-Amino-5-R-1,3,4-thiadiazole derivatives have also been investigated for their ability as complexation agents to obtain compounds with enhanced biological properties due to the presence of metal ions. Thus, 5-(2-aminoethyl)-2-amino-1,3,4-thiadiazole **21** and its Cu(II) and Ni(II) complexes **22a-b** 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

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 al⁴¹ 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 al⁷⁸ 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.

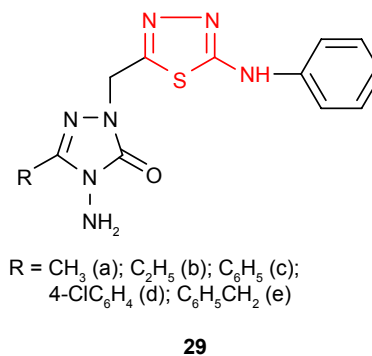


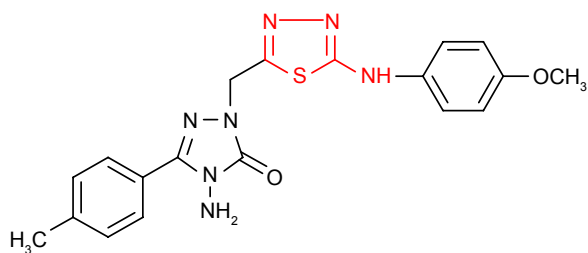
An attempt to prepare active compounds of the thiadiazole derivative series combining the xylosyl moiety and the aryltetrazole ring gave compound **27** exhibiting *S. aureus* inhibitory activity.⁷⁹ It is well known that furan derivatives bearing a nitro group in the 5-position and a group of the general type $-C=N-N=C-$, which may be incorporated in a heterocycle, possess in vivo antibacterial properties. Sherman⁸⁰ 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.

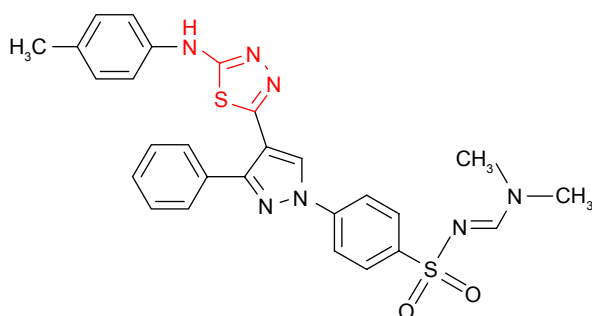
Demirbas et al⁸¹ have synthesized several 1-(5-arylamino-[1,3,4]thiadiazol-2-yl)methyl-4-amino-3-R-5-oxo-4,5-dihydro-[1,2,4]triazoles **29** and **30** which exhibited antimicrobial properties on different microbial strains. *p*-Chlorophenyl derivative **29d** and *p*-methoxyphenylamino derivative **30** 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 **29e** 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 *Penicillium* sp. (diameter of the inhibition zone 20 mm). No antifungal activity was observed on the yeasts such as *C. albicans*, *Candida tropicalis* or *Candida glabrata*. The substitution of the $-NH-$ group in arylamino unit by a hydrophobic methyl group decreased the antimicrobial activity.^{82,83}



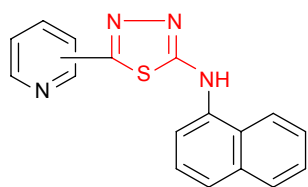


30

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.⁸⁴ Among the studied compounds, the tolyl derivative **31** 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 **32** exhibited very weak inhibitory activity against *S. aureus* and *E. coli*, while the benzyl derivative **33** was more

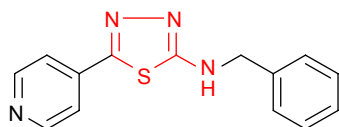


31



2-pyridyl, 3-pyridyl, 4-pyridyl

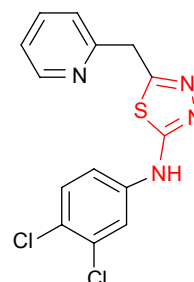
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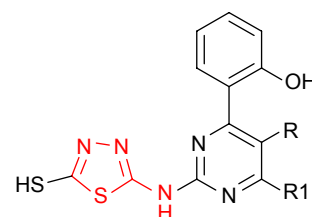
33

active than other studied compounds against tested Gram-positive and Gram-negative bacterial strains.^{85,86}

Similar hybrid compounds which have a 2-pyridyl ring linked to 1,3,4-thiadiazole moiety through a methylene group (eg, compound **34**) also exhibited moderate activity against some Gram-positive cocci, Gram-negative rods and fungi.⁸⁷ Some 1,3,4-thiadiazole derivatives such as **35** bearing pyrimidine ring have been tested for in vitro antimicrobial 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.⁸⁸



34

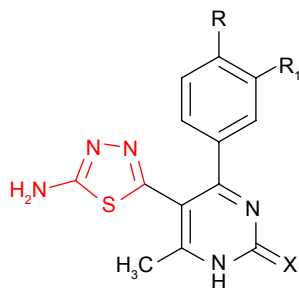
R = CH₃, Cl;R₁ = 2-furyl (a,b); 2-thienyl (c,d); 2-pyridyl (e,f)

35

Andrews et al.⁸⁹ 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*.

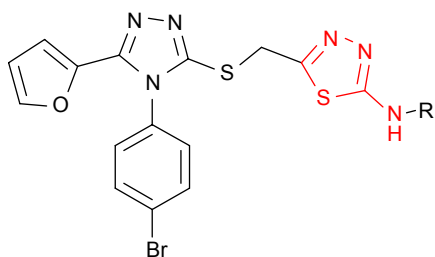
New 2-[4-(4-bromophenyl)-5-(2-furyl)-4H-1,2,4-triazole-3-yl]mercaptomethyl-5-alkyl/arylamino-1,3,4-thiadiazoles **37** were synthesized and have been evaluated for in vitro antifungal activity against *Microsporium gypseum*, *Microsporium canis*, *T. mentagrophytes*, *Trichophyton rubrum* and

C. albicans. Both types of compounds bearing aliphatic and aromatic substituents inhibited the growth of fungal strains at concentrations ranging between 4 and 8 $\mu\text{g/mL}$, the most susceptible microorganism being *T. mentagrophytes* (MIC value of 4 $\mu\text{g/mL}$).⁹⁰



X = O, S
R = OH, R₁ = H (a, b)
R = H, R₁ = NO₂ (c, d)

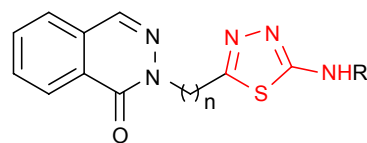
36



R = C₂H₅; C₄H₉; C₆H₄F(*p*); C₆H₄NO₂(*p*)

37

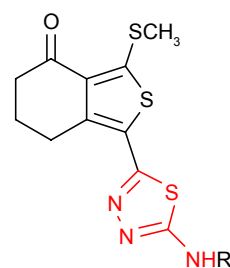
Onkol et al⁹¹ have synthesized several 2-[[1(2*H*)-phthalazine-2-yl]methyl/ethyl]-5-arylamino-1,3,4-thiadiazole derivatives such as **38**. The antimicrobial studies showed that the compounds are active against *B. subtilis* strains and two yeast-like fungi: *C. albicans* and *Candida parapsilosis*. 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(5*H*)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.⁹²



n = 1, 2

R = phenyl; benzyl; phenethyl;
4-ClC₆H₄; 4-CH₃OC₆H₄;
4-CH₃C₆H₄

38

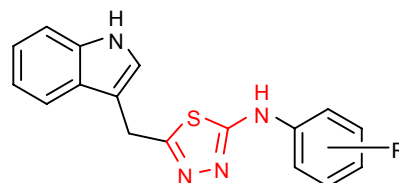


R = CH₃ (a); C₂H₅ (b); *n*-C₄H₉ (c); 4-NO₂C₆H₄ (d)

39

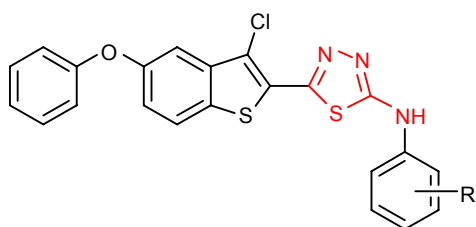
Siddiqui and Alam⁹³ and Siddiqui et al⁹⁴ have synthesized some new 5-(1*H*-indol-3-ylmethyl)-*N*-(substituted phenyl)-1,3,4-thiadiazol-2-amine derivatives such as **40**. The antibacterial and antifungal studies showed good to high activity against *E. coli* and *C. albicans*, respectively, for some of the analyzed compounds.

Vasoya et al⁹⁵ reported the synthesis and antimicrobial screening of new 2-(3'-chloro-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 $\mu\text{g/mL}$. 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



R = H; 2-Cl; 3-Cl; 4-Cl; 2-OCH₃

40

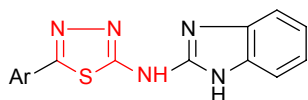


R = H (a); 3-Cl (b); 4-Cl (c); 4-CH₃ (d);
4-CH₃O (e); 2-NO₂ (f)

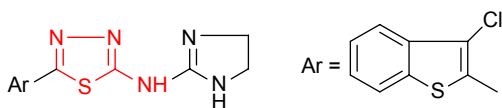
41

electron-donating groups (eg, methyl and methoxy) on C-2 of the arylamino group.

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*.⁹⁶

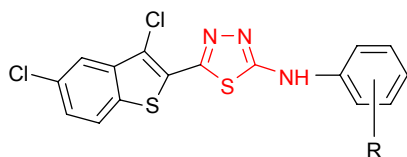


42



43

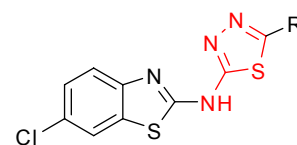
2-(3',5'-Dichlorobenzo[*b*]thiophen-2'-yl)-5-arylamino-1,3,4-thiadiazole derivatives **44** exhibited antimicrobial activity against bacterial and fungal strains. The SAR study showed that the arylamino substituent at C-5 of the thiadiazole ring can change the antimicrobial spectrum. Thus, the nitro derivative **44c** exhibited 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*.⁹⁷



R = 2-CH₃ (a); 4-CH₃ (b); 2-NO₂ (c)

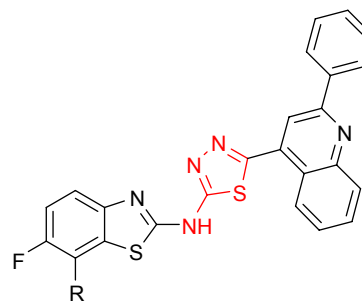
44

Amir et al³⁹ 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-dichlorophenyl (**45c**) groups at C-2 of thiadiazole ring showed potent antibacterial activity, while the compounds having 2-aminophenyl (**45d**) and 2,4-dichlorophenoxyethyl (**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).^{55,98}



R = 2-acetoxyphenyl (a); 2-naphtylmethyl (b);
2,4-dichlorophenyl (c); 2-aminophenyl (d);
2,4-dichlorophenoxyethyl (e)

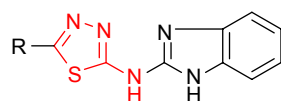
45



R = morpholinyl; piperazinyl

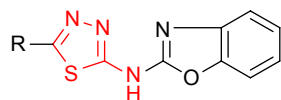
46

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.⁹⁹



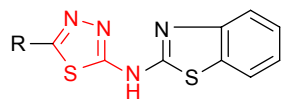
R = benzyl (a); 3-pyridyl (b)

47



R = benzyl (a); 3-pyridyl (b)

48

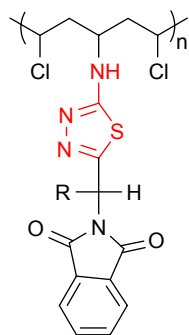


R = benzyl (a); 3-pyridyl (b)

49

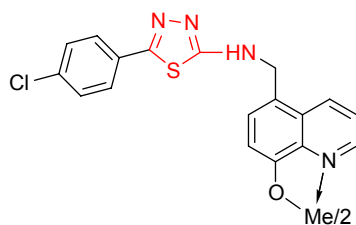
The synthesis of new polymers derived from poly(vinyl chloride) (PVC) was carried out by Yousif et al.¹⁰⁰ The modified PVC polymers **50** contain 1,3,4-thiadiazole ring and were obtained through the chlorine displacement reaction from PVC with heterocyclic moiety. The preliminary screening for the antimicrobial activity of the polymers was performed on three bacterial strains: *S. aureus*, *Streptococcus viridans* and *E. coli* and three fungal strains: *Fusarium oxysporum*, *Alternaria alternata* and *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 **51** was synthesized by Patel and Singh.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. Cu²⁺ chelate **51a** was the most active among the studied compounds.



R = CH₂C₈H₅; CH₂CH₂SCH₃;
C₃H₇ (i); CH₃; H

50

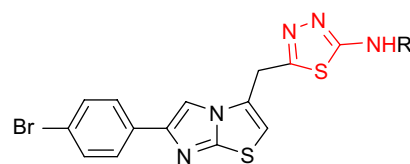


Me = Cu²⁺ (a); Ni²⁺ (b); Co²⁺ (c);
Zn²⁺ (d); Mn²⁺ (e)

51

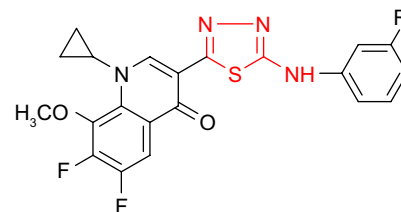
The in vitro activity of 1,3,4-thiadiazole derivatives **52** bearing an imidazo[2,1-*b*]thiazole moiety has been evaluated against bacterial strains of *S. aureus*, *P. aeruginosa* and *E. coli* and fungal strains of *C. albicans*, *C. parapsilosis*, *Candida krusei*, *T. mentagrophytes*, *Trichophyton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

Hybrid molecules **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 **53a** was more active against *A. niger* than nystatin, and the tolyl derivative **53b** was more active against *P. aeruginosa* than chloramphenicol. Compared to the known antibacterial fluoroquinolones, the hybrid compounds **53a-b** showed a very narrow antimicrobial spectrum.¹⁰³



R = CH₃ (a); C₂H₅ (b)

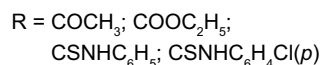
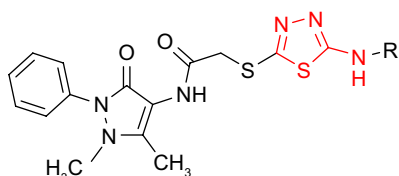
52



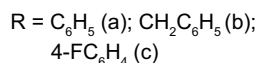
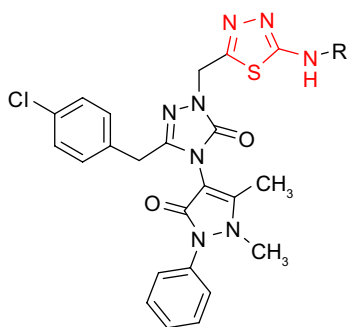
R = H (a); CH₃ (b)

53

The hybrid derivatives **54** having the 1,3,4-thiadiazole ring attached to the antipyrene moiety showed weak inhibitory activity against Gram-positive bacteria *S. aureus* and *B. subtilis* and no activity against some Gram-negative bacteria.¹⁰⁴ However, the introduction of a third heterocyclic nucleus in the structure of some hybrid antipyrene derivatives gave the compounds such as **55** with very good antimicrobial activity against enteric bacteria such as *E. coli*, *Enterobacter aerogenes* and *Y. pseudotuberculosis* and Gram-negative bacteria *P. aeruginosa* (inhibition zone >20 mm at 5 μg/mL). The most active derivative **55a** was two times more active than ampicillin.¹⁰⁵



54

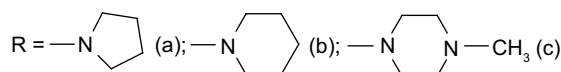
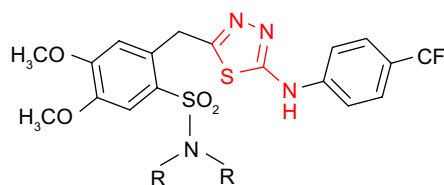


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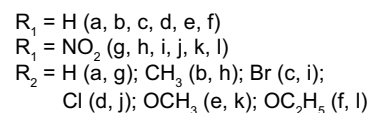
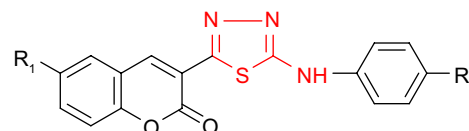
Hybrid sulfonamide-1,3,4-thiadiazole derivatives such as **56**, combining two pharmacologically active molecules in one new compound, have been synthesized by Camoutsis et al.¹⁰⁶ The antimicrobial study against Gram-positive bacteria, Gram-negative bacteria and fungi has revealed some differences depending 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 CF₃ 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,^{107–109} and the antimicrobial 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 µg/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*.



56



57

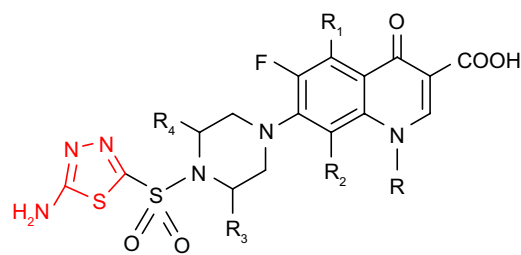
Antitubercular activity

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, sparfloxacin and moxifloxacin) being also used in tuberculosis treatment in the cases involving resistance or intolerance to first-line antituberculosis therapy.^{110,111}

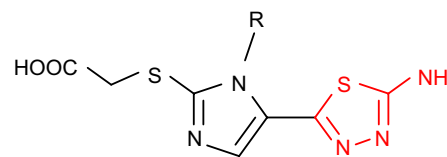
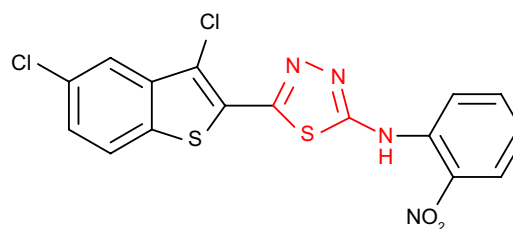
A new class of antibacterial fluoroquinolones is represented by sulfonyl-fluoroquinolones which are hybrid molecules incorporating a sulfonamide moiety and a fluoroquinolone ring. Sulfonyl-fluoroquinolones are particularly active against Gram-positive bacteria. Thus, Talath and Gadad¹¹² have synthesized 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, sparfloxacin 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

**58**

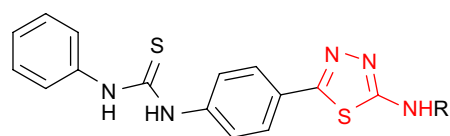
	R	R ₁	R ₂	R ₃	R ₄
a		H	H	H	H
b	C ₂ H ₅	H	H	H	H
c		NH ₂	F	CH ₃	CH ₃
d		H	OCH ₃	H	CH ₃

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-ylthio] 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

R = CH₃; CH₂C₆H₅**59****44c**

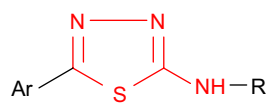
Karakus and Rollas¹¹⁷ have synthesized new *N*-phenyl-*N'*-[4-(5-alkyl/aryl-amino-1,3,4-thiadiazol-2-yl)phenyl]thioureas **60**. Using BACTEC 460 radiometric system (Becton Dickinson, Cockeysville, MD, USA), the in vitro activity against *M. tuberculosis* strain H₃₇Rv at 6.25 µg/mL has been studied. 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. tuberculosis*. Other derivatives exhibited different degrees of inhibition (16% for compound **60c**, 6% for compound **60d**).



R = C₆H₁₁ (a); 4-ClC₆H₄ (b); CH₂C₆H₅ (c); C₂H₅ (d)

60

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. tuberculosis* H₃₇Rv at a concentration of 6.25 µg/mL. Other studied molecules were classified as high activity compounds (28 derivatives, inhibition activity ≥35%), and this structure may

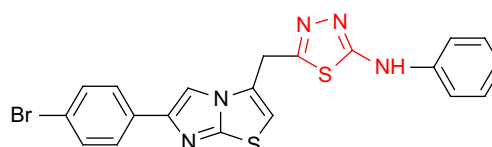


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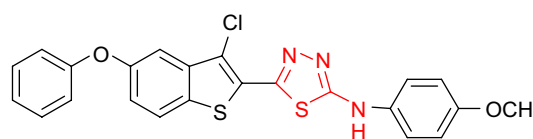
	Ar	R	Inhib (%)
a	C ₆ H ₅	C ₆ H ₅	65
b	C ₆ H ₅	4-FC ₆ H ₄	50
c	4-ClC ₆ H ₄	C ₆ H ₅	50
d	4-ClC ₆ H ₄	4-FC ₆ H ₄	54
e	4-FC ₆ H ₄	C ₆ H ₅	69
f	4-FC ₆ H ₄	4-FC ₆ H ₄	52
g	4-C ₅ H ₄ N	4-BrC ₆ H ₄	53
h	4-C ₅ H ₄ N	4-ClC ₆ H ₄	59

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.⁴²

The antimycobacterial activity against *M. 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 µg/mL).¹⁰² Vasoya et al⁹⁵ have reported the antitubercular activity against *M. 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 µg/mL).



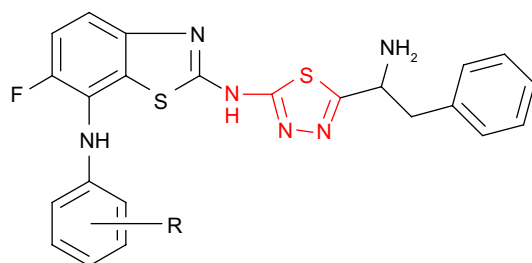
62



41e

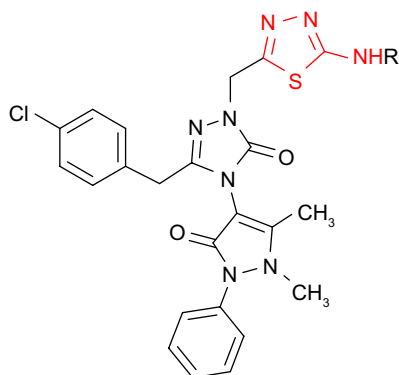
New *N*-[5-(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-7-substituted 1,3-benzothiazol-2-amine derivatives such as **63** have been in vitro evaluated for antitubercular activity against *M. tuberculosis* strain H₃₇Rv. The compounds showed promising antitubercular activity in comparison to standard drugs, rifampicin and isoniazid.¹¹⁸ Bayrak et al¹⁰⁵ 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.



R = *o*, *m*, *p*-NO₂; *o*, *m*, *p*-CH₃O; *o*-Cl

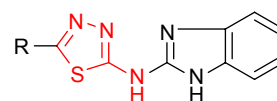
63



R = C₆H₅ (a); CH₂C₆H₅ (b);
4-FC₆H₄ (c)

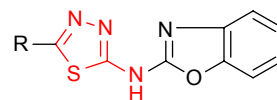
55

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**).⁹⁹



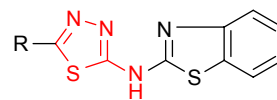
R = benzyl (a); 3-pyridyl (b)

47



R = benzyl (a); 3-pyridyl (b)

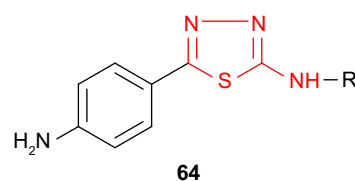
48



R = benzyl (a); 3-pyridyl (b)

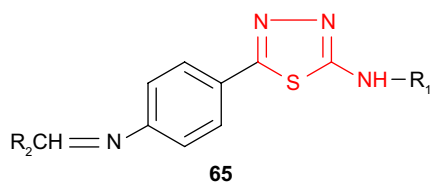
49

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),¹¹⁹ which is in agreement with other studies indicating that the conjugation of hydroxyl ligand with the imine group synergistically enhances the antimycobacterial activity.¹²⁰



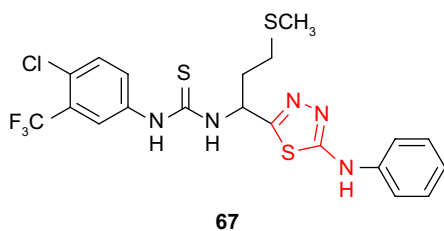
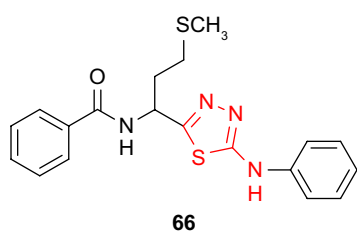
64

	R ₁	Inhib (%)
a	CH ₃	29
b	C ₂ H ₅	34
c	C ₆ H ₅	16
d	4-CH ₃ C ₆ H ₄	22



	R ₂	R ₁	Inhib (%)
a	2-HOC ₆ H ₄	C ₂ H ₅	0
b	3-NO ₂ C ₆ H ₄	C ₂ H ₅	0
c	2-HOC ₆ H ₄	C ₆ H ₅	51
d	3-NO ₂ C ₆ H ₄	C ₆ H ₅	29
e	2-HOC ₆ H ₄	4-CH ₃ C ₆ H ₄	36
f	3-NO ₂ C ₆ H ₄	4-CH ₃ C ₆ H ₄	34

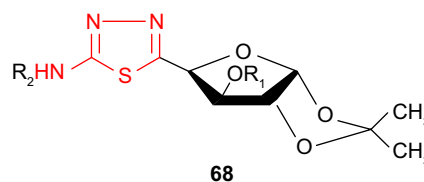
Starting from L-methionine, Tatar et al¹²¹ synthesized different phenylamino-1,3,4-thiadiazole derivatives. 1,3,4-Thiadiazole benzamide **66** and 1,3,4-thiadiazole thiourea **67** exhibited antimycobacterial activity against *M. tuberculosis* H₃₇Rv strain with MIC values of 79.50 and 61.77 μM, respectively. Unfortunately, the compounds proved low selectivity with minimum cytotoxic concentration (MCC) values lower than MIC values.



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.^{122,123} Some sulfide, sulfoxide and sulfonamide thiadiazoles were also reported for their fungicidal activity against *Gibberella zae*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *F. oxysporum* or *Cytospora mandshurica*.⁶ In this connection, various derivatives containing 2-amino-1,3,4-thiadiazole

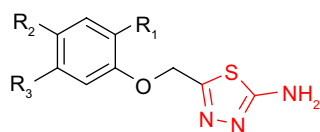
system have been prepared. 2-Arylamino-5-glycosyl-1,3,4-thiadiazole derivatives such as **68** were synthesized by Zong et al,¹²⁴ and the fungicidal activity against six fungal species: *S. sclerotiorum*, *Phytophthora parasitica*, *B. cinerea*, *Rhizoctonia solani*, *Pyricularia oryzae* and *Phoma asparagi* was in vitro investigated. Most of the 20 studied compounds displayed good fungicidal activities against *S. sclerotiorum* (12 compounds with inhibition rate of ≥85%) and *P. oryzae* (seven compounds with inhibition rate of ≥80%) at a concentration of 50 μg/mL. Further studies against *S. sclerotiorum* at the concentrations of 50, 20, 10, 5 and 2 μg/mL were used to determine the effective concentration values EC₅₀. About half of the studied compounds displayed good fungicidal activity with EC₅₀ < 3 μg/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** (EC₅₀ = 0.29 μg/mL) and **68j** (EC₅₀ = 0.46 μg/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 moderate to 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**).¹²⁴



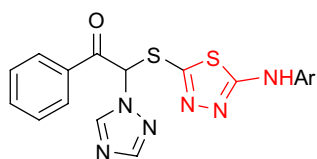
	R ₁	R ₂	EC ₅₀
a	COCH ₃	2,4-(CH ₃) ₂ C ₆ H ₃	7.11
b	COCH ₃	3,4-Cl ₂ C ₆ H ₃	0.29
c	COCH ₃	2,5-Cl ₂ C ₆ H ₃	3.94
d	COCH ₃	1-Naphthyl	22.53
e	COCH ₃	4-Cl-3-CF ₃ C ₆ H ₃	1.50
f	allyl	2,4-(CH ₃) ₂ C ₆ H ₃	9.92
g	allyl	3,4-Cl ₂ C ₆ H ₃	1.50
h	allyl	2,5-Cl ₂ C ₆ H ₃	1.62
i	allyl	1-Naphthyl	2.00
j	allyl	4-Cl-3-CF ₃ C ₆ H ₃	0.46

Yadav et al¹²⁵ have synthesized 2-amino-5-substituted-1,3,4-thiadiazoles **69**, and the *in vitro* fungicidal activity against *P. oryzae* and *R. solani* at 500 ppm was investigated. Fungicidal screening data indicated a moderate activity compared to the reference fungicide, carbendazim, the tested compounds being more active against *P. oryzae* than *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 (*P. oryzae* inhibition rate between 32% and 42%) than the methylated ones (eg, **69c**, *P. oryzae* inhibition rate of 16%), the *para* chlorinated compound **69a** being the most active. Methylation of the *p*-chloroderivative decreased the activity on both fungi.

Chu et al¹²⁶ have synthesized several ω -(5-arylamino-1,3,4-thiadiazol-2-thio)- ω -(1*H*-1,2,4-triazol-1-yl)acetophenone 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.



- R₁ = H, R₂ = Cl, R₃ = H (a)
 R₁ = Cl, R₂ = H, R₃ = H (b)
 R₁ = CH₃, R₂ = H, R₃ = H (c)
 R₁ = H, R₂ = Cl, R₃ = CH₃ (d)

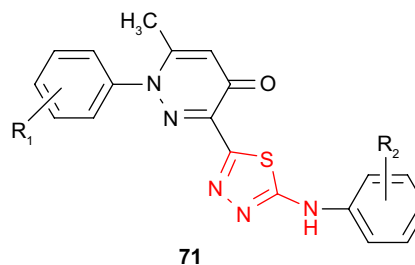
69

- Ar = *m*-CH₃C₆H₄, C₆H₅, *m*-ClC₆H₄, *o*-CH₃C₆H₄

70

In addition, pyridazinone-substituted 1,3,4-thiadiazoles **71** synthesized by Zou et al^{127,128} were tested for *in vivo* antifungal activity against wheat leaf rust, *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 R₂ leads to an increased fungicidal activity (*m*-CF₃ > *o*-F > H). Concerning the substituent R₁, the best and consistent results were obtained in the cases of *ortho* substituents with inductive electron-donating properties. The preferred substituent for R₁ was the 2,4-dimethyl group, as can be seen with compounds **71d** and **71e**.

**71**

	R ₁	R ₂	Inhib (%)
a	2,4-Cl ₂	<i>m</i> -CF ₃	90
b	2,4-Cl ₂	<i>o</i> -F	80
c	2,4-Cl ₂	H	20
d	2,4-(CH ₃) ₂	<i>m</i> -CF ₃	90
e	2,4-(CH ₃) ₂	<i>o</i> -F	90

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.

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