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Dedicated to Professor Mircea Diudea on the Occasion of His 65th Anniversary

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 5-AMINO-2-MERCAPTO-1,3,4-THIADIAZOLE DERIVATIVES THIOETERS AND SCHIFF BASES

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ABSTRACT. Starting from 5-amino-2-mercapto-1,3,4-thiadiazole, 23 compounds, Schiff bases and S-mercapto-substituted derivatives, were synthesized. Their structural elucidation was based on elemental analysis, mass spectrometry and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). The screening of the antimicrobial activity of the title compounds was realized using the diffusimetric method against several strains of Gram-positive and Gram-negative bacteria and one fungal strain (*Candida albicans*). Some of the molecules showed moderate to good antibacterial activity against Gram-negative (*S. typhimurium, E. coli)* and better activity against Gram-positive (*B. cereus*, *L. monocytogenes*, *S. aureus)* bacterial strains. All compounds exhibited moderate to very good activity against *C. albicans*. Qualitative relationships (SAR) were also established between the chemical structures and the antimicrobial activity of these compounds.

Keywords: *1,3,4-thiadiazole, antibacterial activity, antifungal activity.*

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INTRODUCTION

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics in human. The widespread use and misuse of antibiotics led to a serious public health problem: bacterial resistance to antibiotics [1].

According to WHO (World Health Organization), a great percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) or other so called „super bacteria" (*Clostridium difficile*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Serratia* spp. etc.) with enhanced morbidity and mortality, being capable of surviving the effects of most, if not all, antibiotics currently in use, due to multiple mutations [2]. In addition, the theme of the World's Health Day on the 7th of April 2011 was the antimicrobial resistance, showing that this is a major worldwide problem.

On the other hand, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients [3]. The difficulties in developing safe and efficient antifungal are the easily gained resistance and the biochemical similarity of the human cell and fungi, which is also a problem for selective activity [4].

Therefore, the treatment of infectious diseases still remains an important and challenging problem due to a combination of factors including emerging infectious diseases and the dramatically increasing number of multi-drug resistant microbial pathogens [5].

This fact created in the last decades a substantial medical need for new classes of antibacterial agents. It is necessary to develop new approaches of antimicrobial resistance. In this purpose a potential method is to design novel, potent and unique molecules that can be involved in effective therapies with no cross-resistance [6].

Several five membered heteroaryl systems with three heteroatoms at symmetrical positions, such as 1,3,4-thiadiazole, have attracted continuous interest over the years due to their interesting pharmacological activities [7-9].

1,3,4-Thiadiazole is a versatile framework that acts as a "hydrogen binding domain" and as a "two- electron donor system" [10]. It is a basic pharmacophore for a wide variety of biological activities, including antibacterial properties. 1,3,4-Thiadiazole can behave as the bio-isosteric replacement of the thiazole moiety, which can be found in the structure of third and fourth generation cephalosporins. Therefore, 1,3,4-thiadiazole can be successfully used in antibiotic preparations [10,11].

The most studied regioisomeric form of the thiadiazole series, is 1,3,4-thiadiazole and its dihydro-derivatives [12,13]. These heterocyclic systems constitute the active part of several biologically active compounds (Acetazolamide, Methazolamide, Sulfamethizole [7,8], Glybuzole [14] etc.) and exhibits a wide range of therapeutic activities such as antimicrobial [15-20], diuretics [7], anti-leishmanial [21], antiulcer, anti-mycobacterial [15], anti-inflammatory [22], free radical scavenging [23], anticonvulsant [24,25], anticancer [26] and antidepressant [15].

In addition, some *N*-substituted Schiff bases bearing aryl groups or heterocyclic motifs possess excellent biological activities [27,28]. Therefore these new generations of molecules would represent a fruitful matrix for further development of better medicinal agents, being a prevalent scaffold in antimicrobials drugs discovery.

These findings prompted us to prepare and investigate potentially active new antimicrobial agents. We report herein, the synthesis of various new C-2, -5 disubstituted 1,3,4-thiadiazole derivatives and the screening of their antibacterial and antifungal activities, with the aim of having improved activity and dropped toxicity. We also discuss the structure-activity relationship, which can serve as an important tool for medicinal chemists in order to develop better agents in terms of efficacy and safety.

RESULTS AND DISCUSSION

Chemistry. The target compounds, *S*-substituted 5-amino-2-mercapto-1,3,4-thiadiazoles (**2a-f, 3a-b, 4a-e**) and Schiff bases (*N*-substituted 5-mercapto-1,3,4-thidiazol-2-imines) (**5a -d, 6a-d, 7a,b**) were prepared following the routes shown in Schemes 1 and 2. Two of the Schiff bases obtained - **5b** [29] and **5d** [30] - have been previously reported.

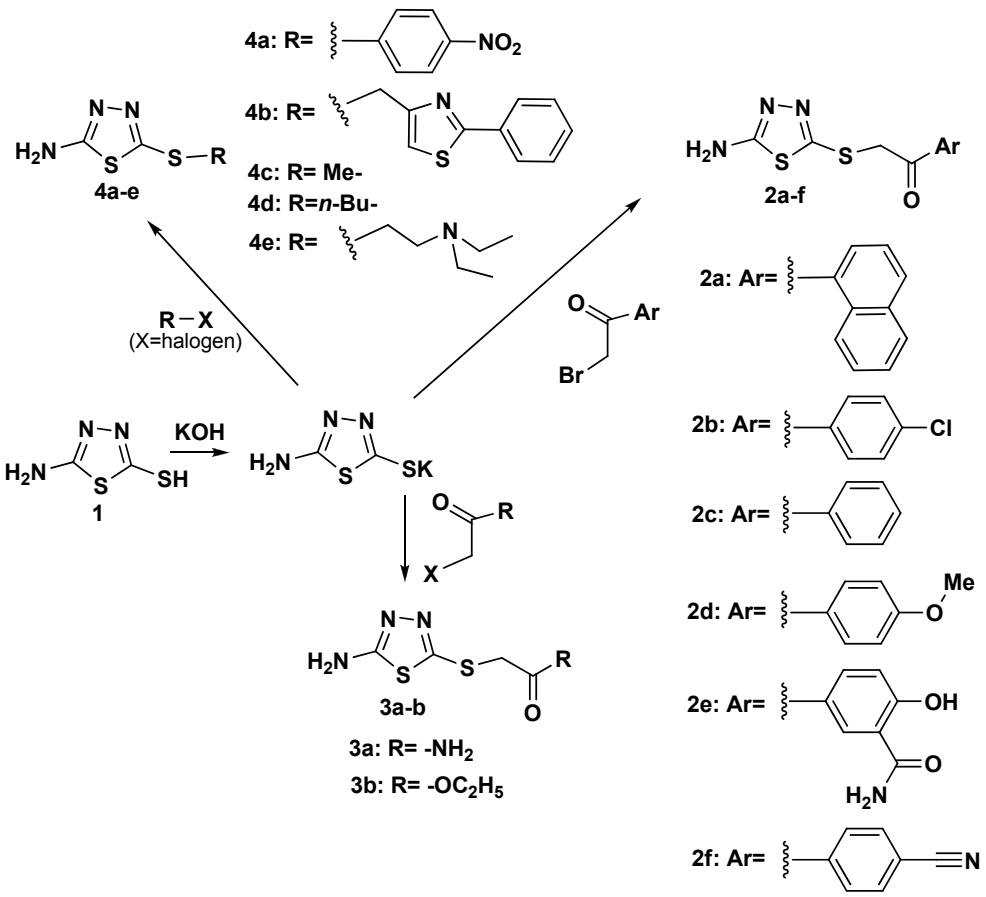
The thioethers **2a-f, 3a-b** and **4a-e** were easily obtained via the potassium salt of the commercial 5-amino-5-mercapto-1,3,4-thiadiazole, by *S*-alkylation in aq. EtOH solutions at 0 °C.

All alkylating agents RX were commercial, except for 4- (iodomethyl)-2-phenylthiazole, whose synthesis was performed according to literature [31].

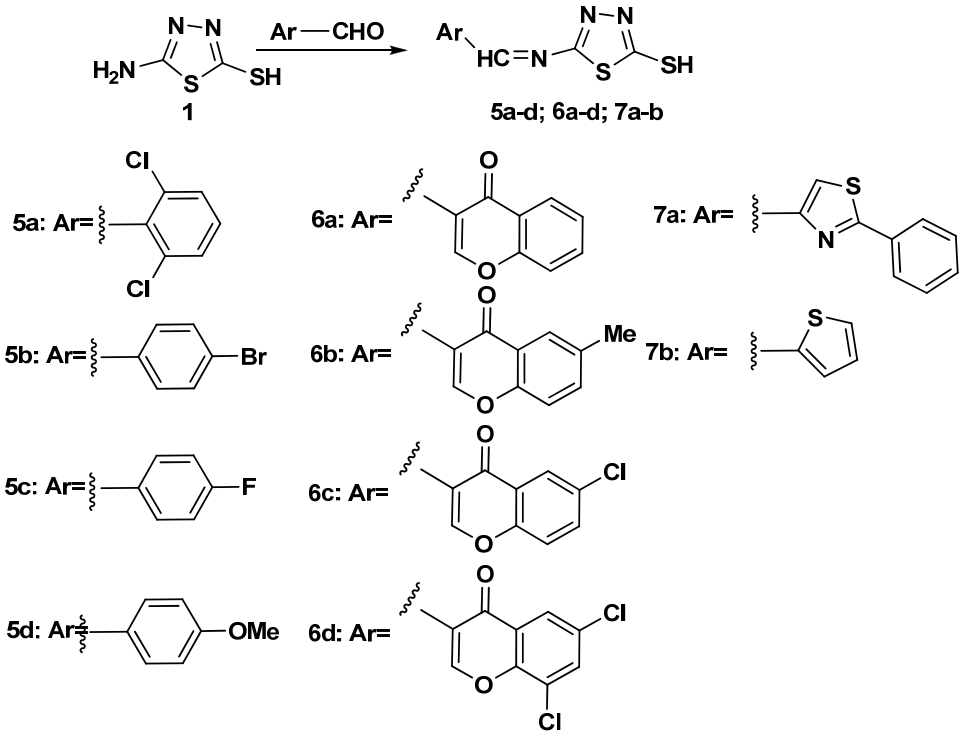
The Schiff bases **5a-d**, **6a-d** and **7a-b** were synthesized by condensation of 5-amino-2-mercapto-1,3,4-thiadiazole **1** with various aromatic aldehydes, in absolute EtOH, in the presence of AcOH as catalyst. The reactions were performed parallelly under reflux conditions, respectively using microwave irradiation. The second method presented several advantages such as: higher yields, shorter reaction durations, reduced amounts of solvents.

All aromatic aldehydes were commercial, with the exception of 2-phenylthiazole-4-carbaldehyde, which was synthesized according to literature [32].

All compounds were characterized by melting point, elemental analysis and spectroscopic data (^{1}H-NMR and MS) which fully confirmed the proposed structures.



Scheme 1. Synthesis of S-substituted 5-amino-2-mercapto-1,3,4-thiadiazoles



Scheme 2. Synthesis of Schiff Bases

Antimicrobial activity. *In vitro* antimicrobial activity was investigated by means of agar disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The antibacterial activity of newly synthesized compounds was evaluated against various pathogenic Gram-negative (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922) and Gram-positive (*Listeria monocytogenes* ATCC 35152, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 13061) bacterial strains. The antifungal activity of the above compounds was evaluated against a strain of *Candida albicans* ATCC 90028.

The results of antifungal and antibacterial activity of *S*-substituted derivatives and Schiff bases are reported in Table 1, in comparison with Ciprofloxacin and Fluconazole, as reference drugs.

Table 1. Antimicrobial activity of compounds **2 to 7**

Compound	Diameter of the inhibition zone (mm)					
	I	II	III	IV	V	VI
2a	18	14	12	12	10	22
2b	18	10	12	10	12	22
2c	18	14	10	12	12	22
2d	14	14	16	12	10	16
2e	14	10	14	12	10	18
2f	14	12	16	10	12	20
3a	12	10	10	16	12	18
3b	12	12	10	14	10	18
4a	12	12	14	10	10	18
4b	16	16	16	12	14	18
4c	14	10	14	14	10	16
4d	16	12	10	14	14	18
4e	16	10	12	12	14	16
5a	16	14	16	16	24	24
5b	18	18	18	20	22	18
5c	26	20	16	18	20	26
5d	20	20	16	18	18	26
6a	26	20	20	28	22	24
6b	20	16	22	24	26	30
6c	22	22	24	18	26	28
6d	22	18	22	26	26	18
7a	20	20	18	20	20	22
7b	22	18	16	20	22	28
C	26	12	16	26	14	-
F	-	-	-	-	-	28

- Agar diffusion technique, diameter of inhibition (mm). Solutions of compounds: 1 mg/mL (DMSO) (50 µl/well). **Microbial strains I** = *Salmonella typhimurium* ATCC 13311; **II** = *Escherichia coli* ATCC 25922; **III** = *Bacillus cereus* ATCC 13061; **IV** = *Listeria monocytogenes* ATCC 35152; **V** = *Staphylococcus aureus* ATCC 25923; **VI** = *Candida albicans* ATCC 90028; **C**= Ciprofloxacin, **F**= Fluconazole.

All the tested thioethers showed a moderate activity against Gram-positive and Gram-negative bacterial strains, however a moderate to good antifungal activity. The most active compounds against *Salmonella typhimurium* and *Candida albicans* were those of series **2**, **2a-c** being the best. Therefore, the antifungal and the anti-*Salmonella* activity in the series of thioethers increased when the compounds were *S*-substituted with a 1-arylethanone group, especially in the case of unsubstituted or halogenated aryl-derivatives.

All the tested Schiff bases showed a moderate to good antibacterial activity and a good to very good antifungal activity. The antibacterial activity was significantly increased against Gram-positive bacterial strains.

In the case of compounds **5a-d**, *para*-substitution was found to be favorable for the antimicrobial activity, except for *Staphylococcus aureus*, against which the *ortho,ortho'*-disubstituted compound **5a** proved to be more active. In addition, in the series of 4-halo derivatives a higher activity against Gram-negative bacterial strains was observed for compounds substituted with the most electronegative halogen (**5c** (4-F) > **5b** (4-Br)). The best antibacterial activity against Gram-positive was detected for compounds substituted with the least electronegative halogen (**5b** (4-Br) > **5c** (4-F)).

The best antimicrobial activities in the series of Schiff bases were displayed for compounds **6a-d** possessing a 4*H*-chromen-4-one moiety. The halo-substitution in this zone (**6c-d**) generally increased the antibacterial activity, but di-halo-substitution decreased the antifungal activity. However, compound **6a** with unsubstituted chromene-4-one presented the largest diameters of the zones of inhibition against a Gram-negative (*Salmonella typhimurium*) and a Gram-positive bacteria strain (*Listeria monocytogenes*). The activity against Gram-positive bacteria (except for *Listeria monocytogenes*) was significantly increased in the case of compounds **6b-d** having a substituted chromen-4-one in their structure.

The best antifungal action was observed for the monosubstituted chromen-4-one derivatives, compound **6b** being the most active from all the synthesized molecules. Schiff bases **7a** and **7b** did not show a significant antimicrobial effect, except for **7b** (comprising a thiophene moiety in the structure), which revealed good antifungal properties.

CONCLUSIONS

In conclusion, starting from 5-amino-2-mercapto-1,3,4-thiadiazole, two series of compounds, *S*-substituted derivatives and Schiff bases, have been successfully prepared by *S*-alkylation or condensation with aromatic aldehydes respectively. The microwave irradiation method used in the synthesis of Schiff bases showed better yields than the classical procedure under reflux, as shown in Experimental part.

The antibacterial and antifungal activities were evaluated against several Gram-positive, Gram-negative bacteria and *Candida albicans*. The results indicated that some of the tested molecules show promising antibacterial and antifungal effects. The Schiff bases proved to be more active than the thioethers, representative being the chromene-4-one derivated compounds.

EXPERIMENTAL SECTION

General

Reagents and solvents were purchased from Sigma-Aldrich® and used without further purification. The melting points were measured with an Electrothermal® instrument and are uncorrected. Thin layer chromato-graphy (TLC) used precoated Silica Gel 60F254 sheets (eluent heptane – ethyl-acetate 3:7 v/v, throughout). ^{1}H NMR Spectra were recorded at room temperature on a Bruker® Avance NMR spectrometer operating at 500 MHz. All chemical shifts (δ values) are given in parts per million (ppm) and were measured against the solvent peak. Elemental analysis was performed with a Vario El® CHNS instrument.

Chemistry

General procedure for the synthesis of thioethers (2a-f, 3a-b, 4a-e). 5-amino-2-mercapto-1,3,4-thiadiazole (**1**) (1.33 g, 10 mmol) was suspended in water (10 mL) and a solution obtained by dissolving potassium hydroxide (0.56 g, 10 mmol) in water (5 mL) was added dropwise under vigorous stirring, until complete dissolution of **1**. Ethanol (30 mL) was then added and the mixture was cooled at 0 °C. At this temperature, the corresponding alkylating agent (10 mmol), as a minimum volume of ethanol solution was added dropwise, under vigorous stirring, within one hour. After that, stirring was continued for additional 3 hours. Isolation of the final products was performed as follows:

- in the case of compounds **2a-f**, the resulted suspension was filtered off, washed with water (3 x 50 mL) to provide the crude product.
- in the case of compounds **3a-b** and **4a-e** the ethanol from the reaction mixture was evaporated in vacuum and the resulting aqueous suspension was filtered off and washed with water (3 x 50 mL) to provide the crude product.

After drying, the recrystallization from ethanol yielded the desired compounds as pure analytical samples.

2-(5-Amino-1,3,4-thiadiazol-2-ylthio)-1-(naphthalen-1-yl)ethanone (2a). Synthesis was performed according to the general procedure, using 2-bromo-1-(naphthalen-1-yl)ethanone as the alkylating agent (2.48 g, 10 mmol). Yield (89%), white solid, mp 189-190 °C; ^{1}H NMR (DMSO-*d*6, 500MHz, ppm) *δ*: 8.78 (m, 1H, ArH), 8.13-8.15 (m, 1H, ArH), 7.99-8.06 (m, 3H, ArH), 7.64-7.72 (m, 2H, ArH), 7.32 (s, 2H, -NH2), 4.96 (s, 2H, CH2); MS (EI, 70 eV) *m/z*: 302 (M+1). *Anal*. calcd. (%) for C14H11N3OS2: C, 55.79; H, 3.68; N, 13.94; S, 21.28. Found: C, 55.70; H, 3.74; N, 14.00; S, 21.25.

2-(5-Amino-1,3,4-thiadiazol-2-ylthio)-1-(4-hlorophenyl)ethanone (2b). Synthesis was performed according to the general procedure, using 2-bromo-1-(4-chlorophenyl)ethanone as the alkylating agent (2.32 g, 10 mmol). Yield (87.5%), white solid, mp 192-193 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 8.02-8.04 (m, 2H, Ph), 7.63-7.65 (m, 2H, Ph), 7.32 (s, 2H, -NH_2), 4.80 (s, 2H, CH_2); MS (EI, 70 eV) *m/z*: 286 (M+1). *Anal*. calcd. (%) for $C_{10}H_8ClN_3OS_2$: C, 42.03; H, 2.82; N, 14.70; S, 21.28. Found: C, 41.95; H, 2.76; N, 14.91; S, 21.21.

2-(5-Amino-1,3,4-thiadiazol-2-ylthio)-1-phenylethanone (2c). Synthesis was performed according to the general procedure, using 2-bromo-acetophenone as the alkylating agent (1.98 g, 10 mmol). Yield (82%), white solid, mp 178-179 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.32 (s, 2H, -NH_2), 7.45-7.86 (m, 5H, Ph), 4.81 (s, 2H, CH_2); MS (EI, 70 eV) *m/z*: 252 (M+1). *Anal*. calcd. (%) for $C_{10}H_9N_3OS_2$: C, 47.79; H, 3.61; N, 16.72; S, 25.52. Found: C, 47.65; H, 3.70; N, 16.75; S, 25.55.

(5-Amino-1,3,4-thiadiazol-2-ylthio)-1-(4-ethoxyphenyl)ethanone (2d). Synthesis was performed according to the general procedure, using 2-bromo-1-(4-methoxyphenyl)ethanone as the alkylating agent (2.28 g, 10 mmol). Yield (91%), white solid, mp 191-193 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.99-8.01 (m, 2H, Ph), 7.32 (s, 2H, -NH_2), 7.06-7.08 (m, 2H, Ph), 4.75 (s, 2H, CH_2), 3.86 (s, 3H, -CH_3); MS (EI, 70 eV) *m/z*: 282 (M+1). *Anal*. calcd. (%) for $C_{11}H_{11}N_3O_2S_2$: C, 46.96; H, 3.94; N, 14.93; S, 22.79. Found: C, 46.67; H, 4.06; N, 15.00; S, 22.89.

5-[2-(5-Amino-1,3,4-thiadiazol-2-ylthio)acetyl]-2-hydroxybenzamide (2e). Synthesis was performed according to the general procedure, using 5'-(2-bromoacetyl)-2'-hydroxybenzamide as the alkylating agent (2.57 g, 10 mmol). Yield (95%), white solid, mp 235-257 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.49-8.01 (m, 3H, Ph), 7.34 (s, 2H, -NH_2), 7.20 (s, 2H, $CONH_2$), 5.85 (s, 1H, OH), 4.78 (s, 2H, CH2) ; MS (EI, 70 eV) *m/z*: 311 (M+1). *Anal*. calcd. (%) for $C_{11}H_{10}N_4O_3S_2$: C, 42.57; H, 3.25; N, 18.05; S, 20.66. Found: C, 42.33; H, 3.30; N, 18.20; S, 20.68.

4-[2-(5-Amino-1,3,4-thiadiazol-2-ylthio)acetyl]benzonitrile (2f). Synthesis was performed according to the general procedure, using 4'-(2-bromoacetyl)benzo-nitrile (2.24 g, 10 mmol) as the alkylating agent. Yield (80%), white solid, mp 183 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 8.15-8.17 (m, 2H, Ph), 8.04-8.06 (m, 2H, Ph), 7.32 (s, 2H, -NH_2), 4.84 (s, 2H, CH_2); MS (EI, 70 eV) *m/z*: 277 (M+1). *Anal*. calcd. (%) for $C_{11}H_8N_4OS_2$: C, 47.81; H, 2.92; N, 20.27; S, 23.21. Found: C, 47.92; H, 2.80; N, 20.36; S, 23.11.

2-(5-Amino-1,3,4-thiadiazol-2-ylthio)acetamide (3a). Synthesis was performed according to the general procedure, using 2-chloroacetamide as the alkylating agent (0.93 g, 10 mmol), in the presence of a spatula tip of KI. Yield (84%), white solid, mp 213-215 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.34 (s, 2H, -NH_2), 7.18 (s, 2H, $CONH_2$), 4.82 (s, 2H, CH_2); MS (EI, 70 eV) *m/z*: 191 (M+1). *Anal*. calcd. (%) for $C_4H_6N_4OS_2$: C, 25.25; H, 3.18; N, 29.45; S, 33.71. Found: C, 25.12; H, 3.28; N, 29.58; S, 33.61.

Ethyl 2-(5-amino-1,3,4-thiadiazol-2-ylthio)acetate (3b). Synthesis was performed according to the general procedure, using ethyl bromoacetate as the alkylating agent (1.66 g, 10 mmol). Yield (74%), white solid, mp 83-84°C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.36 (s, 2H, -NH_2), 4.74 (q, 2H, CH_2), 4.72 (s, 2H, CH_2), 1.19 (t, 3H, CH_3); MS (EI, 70 eV) *m/z*: 220 (M+1). *Anal*. calcd. (%) for $C_6H_9N_3O_2S_2$: C, 32.86; H, 4.14; N, 19.16; S, 29.25. Found: C, 33.04; H, 4.00; N, 20.00; S, 29.15.

5-Amino-2-(4-nitrobenzylthio)-1,3,4-thiadiazole (4a). Synthesis was performed according to the general procedure, using 4-nitrobenzyl chloride (1.71 g, 10 mmol) as the alkylating agent. Yield (82%), white solid, mp 165-166 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.75-8.30 (m, 4H, Ph), 7.28 (s, 2H, -NH_2), 4.83 (s, 2H, CH_2); MS (EI, 70 eV) *m/z*: 269 (M+1). *Anal*. calcd. (%) for $C_9H_8N_4O_2S_2$: C, 40.29; H, 3.01; N, 20.88; S, 23.90. Found: C, 40.40; H, 2.94; N, 20.74; S, 23.90.

5-Amino-2-[(2-phenylthiazol-4-yl)methylthio]-1,3,4-thiadiazole (4b). Compund **1** (1.33 g, 10 mmol) was dissolved in ethanol (50 ml) and the reaction mixture was brought to reflux. Potassium carbonate was then added (1.2 eq), and an ethanolic solution of 4-(iodomethyl)-2-phenylthiazole (3 g, 10 mmol) was added dropwise during one hour, under vigorous stirring. Reflux was continued for additional 3 hours, and then the solvent was evaporated under vacuum. The obtained oily residue was extracted with CH_2Cl_2:Et_2O 1:1 (3 x 10 mL), and the solution was dryed over anhydrous sodium sulfate and evaporated under vacuum. The final residue was purified by column cromatography, using silicagel G_{254} and AcOEt:heptane (7:3) as eluent. Yield (76%), white solid, mp 71-73 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ:7.92-7.94 (m, 2H, -NH_2), 7.48-7.50 (m, 5H, Ph), 5.41-5.44 (t, 1H, Thiazole-CH), 4.62-4.64 (dd, 2H, CH_2); MS (EI, 70 eV) *m/z*: 307 (M+1). *Anal*. calcd. (%) for $C_{12}H_{10}N_4S_3$: C, 47.03; H, 3.29; N, 18.28; S, 31.39. Found: C, 46.88; H, 3.35; N, 18.35; S, 31.44.

5-Amino-2-(methylthio)-1,3,4-thiadiazole (4c). Synthesis was performed according to the general procedure, using methyl iodide (1.42 g, 10 mmol) as the alkylating agent. Yield (79.6%), white solid, mp 179-180 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 7.32 (s, 2H, -NH_2), 2.60 (s, 3H, CH_3); MS (EI, 70 eV) *m/z*: 148 (M+1). *Anal*. calcd.(%) for $C_3H_5N_3S_2$: C, 24.47; H, 3.42; N, 28.54; S, 43.56. Found: C, 24.58; H, 3.36; N, 28.47; S, 43.58.

5-Amino-2-(butylthio)-1,3,4-thiadiazole (4d). Synthesis was performed according to the general procedure, using 1-bromobutane (1.36 g, 10 mmol) as the alkylating agent. Yield (76.7%), white solid, mp 121-122 °C; ^{1}H NMR (DMSO-*d*6, 500MHz, ppm) *δ*: 7.34 (s, 2H, -NH2), 2.79-2.82 (t, 2H, CH2), 1.54-1.59 (quin, 2H, CH2), 1.45-1.53 (sextet, 2H, CH2), 0.89-0.92 (t, 3H, CH3); MS (EI, 70 eV) *m/z*: 190 (M+1). *Anal*. calcd. (%) for C6H11N3S2: C, 38.07; H, 5.86; N, 22.20; S, 33.88. Found: C, 37.99; H, 5.78; N, 22.30; S, 33.94.

5-Amino-2-[2-(diethylamino)ethylthio]-1,3,4-thiadiazole (4e). Synthesis was performed according to the general procedure, using 2-chloro-*N,N*-diethylethanamine (1.35 g, 10 mmol) as the alkylating agent. Yield (77%), white solid, mp 140-141 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) *δ*: 7.26 (s, 2H, -NH_2), 3.14 (t, 2H, CH_2), 2.68 (t, 2H, CH_2), 2.45-2.51 (q, 4H, CH_2), 0.92-0.95 (t, 6H, CH_3); MS (EI, 70 eV) *m/z*: 233 (M+1). *Anal*. calcd. (%) for $C_8H_{16}N_4S_2$: C, 41.35; H, 6.94; N, 24.11; S, 27.60. Found: C, 41.49; H, 6.86; N, 24.05; S, 27.62.

General procedure for the synthesis of Schiff bases

Technique 1 **(classic)**:

A solution of 2-amino-5-mercapto-1,3,4-thiadiazole (0.665 g, 5 mmol) in absolute ethanol (20 mL) was warmed to reflux, when the corresponding aromatic aldehyde (5 mmol) and 3 drops of glacial acetic acid were added. The reaction mixture was stirred for 30 minutes at room temperature and then refluxed for 8 hours. The resulted solution was cooled at room temperature then evaporated to a small volume under reduced pressure. The final crystalline suspension was filtered off to provide the crude product. After drying, an additional recrystallisation from methanol:dichloromethane (9:1 v/v) yielded the desired Schiff bases as pure analytical samples.

Technique 2 **(microwave)**:

To an ethanolic solution of 2-amino-5-mercapto-1,3,4-thiadiazole (0.266 g, 2 mmol), the corresponding aromatic aldehyde (2 mmol) and 3 drops of glacial acetic acid were added. The mixture was subjected to microwave for 20 minutes, at 100 °C and 150 W. The work-up of the reaction mixture was identical with that used in *Technique 1.*

5-(2,6-Dichlorobenzylideneamino)-1,3,4-thiadiazole-2-thiol (5a). Synthesis was performed according to the general procedure (technique 1), using 2,6-dichlorobenzaldehyde (0.869 g, 5 mmol) as the aromatic aldehyde. Yield (70%), yellow solid, mp 280 °C; ^{1}H NMR (DMSO-*d*6, 500MHz, ppm) *δ*: 10.00 (s, 1H, SH), 8.88 (s, 1H, OH), 7.11-7.34 (m, 3H, Ph); MS (EI, 70 eV) *m/z*: 290 (M+1). *Anal*. calcd. (%) for C9H5Cl2N3S2: C, 37.25; H, 1.74; N, 14.48; S, 22.10. Found: C, 38.15 ; H, 1.53; N, 13.42; S, 20.52.

5-(4-Bromobenzylideneamino)-1,3,4-thiadiazole-2-thiol (5b) [29]. This compound was synthesized using the both techniques described in the general procedure: under reflux and under microwave irradiation, the last one showing better yields. 4-Bromobenzaldehyde (0.368 g, 2 mmol) was used as the aldehyde for the condensation reaction. Yield (66% - under reflux; 83% - microwave-assisted), yellow solid, mp 215 °C; ^{1}H NMR (DMSO-*d*6, 500MHz, ppm) *δ*: 10.00 (s, 1H, SH), 8.74 (s, 1H, CH), 7.49-7.53 (m, 4H, Ph); MS (EI, 70 eV) *m/z*: 300 (M+1). *Anal*. calcd. (%) for C9H6BrN3S2: C, 36.01; H, 2.01; N, 14.00; S, 21.36. Found: C, 35.90; H, 2.10; N, 14.09; S, 21.29.

5-(4-Fluorobenzylideneamino)-1,3,4-thiadiazole-2-thiol (5c). Synthesis was performed according to the general procedure (technique 1), using 4-fluorobenzaldehyde (0.620 g, 5 mmol) as the aromatic aldehyde. Yield (69%), yellow solid, mp 203 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) *δ*: 9.91 (s, 1H, SH), 8.92 (s, 1H, CH), 7.32-7.45 (m, 4H, Ph); MS (EI, 70 eV) *m/z*: 240 (M+1). *Anal*. calcd. (%) for $C_9H_6FN_3S_2$: C, 45.17; H, 2.53; N, 17.56; S, 26.80. Found: C, 45.10; H, 2.40; N, 17.65; S, 26.91.

5-(4-Methoxybenzylideneamino)-1,3,4-thiadiazole-2-thiol (5d) [30]. Synthesis was performed according to the general procedure (technique 1), using 4-methoxybenzaldehyde (0.680 g, 5 mmol) as the aromatic aldehyde. Yield (67%), yellow solid, mp 199 °C; ^{1}H NMR (DMSO-*d*6, 500MHz, ppm) *δ*: 10.03 (s, 1H, SH), 9.00 (s, 1H, OH), 6.72-7.20 (m, 4H, Ph), 3.89 (s, 3H, CH3); MS (EI, 70 eV) *m/z*: 252 (M+1). *Anal*. calcd. (%) for C10H9N3OS2: C, 47.79; H, 3.61; N,16.72; S, 25.52. Found: C, 47.92; H, 3.51; N, 16.83; S, 25.44.

3-{[(5-Sulfanyl-1,3,4-thiadiazol-2-yl)imino]methyl}-4*H*-chromen-4-one (6a). This compound was synthesized using the both techniques described in the general procedure: under reflux and under microwave irradiation, the last one showing better yields. 4-oxo-4*H*-chromene-3-carbaldehyde (0.348 g, 2 mmol) was used as the aromatic aldehyde. Yield (68% - under reflux; 86% - microwave-assisted), yellow solid, mp 230 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) *δ*: 9.98 (s, 1H, SH), 8.90 (s, 1H, CH), 8.20 (s, 1H, Chromone-CH), 7.57-8.15 (m, 4H, Chromone-CH); MS (EI, 70 eV) *m/z*: 290 (M+1). *Anal*. calcd. (%) for $C_{12}H_7N_3O_2S_2$: C, 49.81; H, 2.44; N, 14.52; S, 22.16. Found: C, 49.74; H, 2.50; N, 14.60; S, 22.09.

6-Methyl-3-{[(5-sulfanyl-1,3,4-thiadiazol-2-yl)imino]methyl}-4*H*-chromen-4-one (6b). This compound was synthesized according to the general procedure in both ways: under reflux and under microwave irradiation. The aromatic aldehyde used was 6-methyl-4-oxo-4*H*-chromene-3-carbaldehyde (0.940 g, 5 mmol – under reflux 0.376 g, 2 mmol – microwave-assisted) Yield (69% - under reflux; 86% - microwave-assisted), yellow solid,

mp 260°C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 10.09 (s, 1H, SH), 9.03 (s, 1H, CH), 8.26 (s, 1H, Chromone-CH), 7.42-7.90 (m, 3H, Chromone-CH), 2.73 (s, 3H, CH_3); MS (EI, 70 eV) *m/z*: 304 (M+1). *Anal*. calcd. (%) for $C_{13}H_9N_3O_2S_2$: C, 51.47; H, 2.99; N, 13.85; S, 21.14. Found: C, 51.35; H, 3.05; N, 13.98; S, 21.07.

6-Chloro-3-{[(5-sulfanyl-1,3,4-thiadiazol-2-yl)imino]methyl}-4*H*-chromen-4-one (6c). This compound was synthesized using the both techniques described in the general procedure: under reflux and under microwave irradiation, the last one showing better yields. 6-chloro-4-oxo-4*H*-chromene-3-carbaldehyde (0.416 g, 2 mmol) was used as the aromatic aldehyde. Yield (65% - under reflux; 82% - microwave-assisted), yellow solid, mp 245 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 10.11 (s, 1H, SH), 8.97 (s, 1H, CH), 8.22 (s, 1H, Chromone-CH), 7.36-7.60 (m, 3H, Chromone-CH); MS (EI, 70 eV) *m/z*: 324 (M+1). *Anal*. calcd. (%) for $C_{12}H_6ClN_3O_2S_2$: C, 44.51; H, 1.87; N, 12.98; S, 19.81. Found: C, 44.70; H, 1.78; N, 12.92; S, 19.77.

6,8-Dichloro-3-{[(5-sulfanyl-1,3,4-thiadiazol-2-yl)imino]methyl}-4*H*-chromen-4-one (6d). The synthesis was performed according to the general procedure, using the both techniques: under reflux and under microwave irradiation. 6,8-Dichloro-4-oxo-4*H*-chromene-3-carbaldehyde (0.484 g, 2 mmol – microwave-assisted) was used as the aromatic aldehyde. Yield (63% - under reflux; 80% - microwave assisted), yellow solid, mp 242 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 10.10 (s, 1H, SH), 9.05 (s, 1H, CH), 8.25 (s, 1H, Chromone-CH), 7.98 (d, 1H, Chromone-CH), 7.81 (d, 1H, Chromone-CH); MS (EI, 70 eV) *m/z*: 358 (M+1). *Anal*. calcd. (%) for $C_{12}H_5Cl_2N_3O_2S_2$: C, 40.23; H, 1.41; N, 11.73; S, 17.90. Found: C, 40.32; H, 1.35; N, 11.78; S, 17.82.

5-{[(2-Phenyl-1,3-thiazol-4-yl)methylidene]amino}-1,3,4-thiadiazole-2-thiol (7a). Synthesis was performed according to the general procedure (technique 1), using 2-phenylthiazole-4-carbaldehyde (0.945 g, 5 mmol) as the aromatic aldehyde. Yield (70%), yellow solid, mp 244 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 10.04 (s, 1H, SH), 9.05 (s, 1H, CH), 7.82 (s, 1H, Thiazole-CH), 7.28-7.43 (m, 5H, Ph); MS (EI, 70 eV) *m/z*: 305 (M+1). *Anal*. calcd. (%) for $C_{12}H_8N_4S_3$: C, 47.35; H, 2.65; N, 18.40; S, 31.60. Found: C, 47.28; H, 2.75; N, 18.32; S, 31.65.

5-[(Thiophen-2-ylmethylidene)amino]-1,3,4-thiadiazole-2-thiol (7b). Synthesis was performed according to the general procedure (technique 1), using thiophene-2-carbaldehyde (0.560 g, 5 mmol) as the aromatic aldehyde. Yield (65%), yellow solid, mp 195 °C; ^{1}H NMR (DMSO-d_6, 500MHz, ppm) δ: 10.01 (s, 1H, SH), 9.02 (s, 1H, CH), 7.00-7.40 (m, 3H, Thiophene-CH); MS (EI, 70 eV) *m/z*: 228 (M+1). *Anal*. calcd. (%) for $C_7H_5N_3S_3$: C, 36.98; H, 2.22; N, 18.48; S, 42.32. Found: C, 36.85; H, 2.32; N, 18.56; S, 42.26.

Antimicrobial activity assay

For antibacterial testing, Mueller-Hinton agar medium was used. For antifungal testing Mueller-Hinton medium supplemented with 2% glucose (providing adequate growth of yeasts) and 0.5 mg/mL methylene blue (providing a better definition of the inhibition zone diameter) was used. The inoculum was prepared by suspending five representative colonies, obtained from an 18–24 h culture on non-selective nutritive agar medium, in sterile distilled water. The cell density was adjusted to the density of a 0.5 McFarland standard by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm and adding sterile distilled water as required (corresponding to a population of 1-5x10^6 CFU/mL). Six-millimeter diameter wells were cut from the agar using a sterile cork-borer, and a predetermined volume of each compound solution was delivered into the wells. A sterile swab was soaked in suspension and then the Mueller-Hinton agar plates were inoculated by streaking the entire surface. After drying for 10-15 minutes, the six millimeter diameter wells were inoculated with 50µl from 10 mg/mL solution in dimethyl sulfoxide (DMSO) (Merck, Germany) of each compound (50 µg/well). Ciprofloxacin (50 µg/well) and Fluconazole (50 µg/well) were used as standard drugs. The plates were incubated at 35°C. Zone diameters were measured to the nearest whole millimeter at a point in which there will be no visible growth after 24 – 48 h. The solvent used for the preparation of the newly synthesized compound solutions, DMSO did not show inhibition against the tested bacterial and fungal strains. Results were obtained in duplicate.

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