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Original article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 3,5-DISUBSTITUTED-TETRAHYDRO-2*H*-1,3,5-THIADIAZINE-2-THIONE DERIVATIVES

Evren SAĞLAM[1], Selma SARAÇ[1], Ekrem KILIÇ[2], Meral ÖZALP[2], Mevlüt ERTAN[1*]

[1]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Ankara, TURKEY
[2] Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06100 Ankara, TURKEY

Abstract

*A series of new 3,5-disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT) derivatives (**4a-g**) were prepared using a convenient and general one-pot procedure and evaluated for their in vitro antibacterial and antifungal activities by using the microdilution method in comparison with ampicillin and fluconazole. 3-Phenyl-5-(1-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4a**) and 3-phenyl-5-hydroxy-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4g**) were found to be active against Staphylococcus aureus and Enterococcus faecalis with MIC values of 4 and 16 µg/mL, respectively. The antifungal activity of 3-phenyl-5-(1-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4a**) against Candida krusei and C. parapsilosis appeared greater than that of fluconazole (MIC: 64 µg/mL and 8 µg/mL) with MIC of 8 and 4 µg/mL, respectively. 3-Phenyl-5-(1-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4a**) also exhibited antifungal activity against C. albicans with a MIC of 4 µg/mL. The antifungal activity of 3-(1-phenylethyl-5-[α-(isobutyl)carboxymethyl]-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4b**) and 3-benzyl-5-carboxyethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4c**) against C. krusei were found to be similar to that of fluconazole (MIC: 64 µg/mL).*

Key words: *3,5-Disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione, Hydroxylamine, Prodrug, Antibacterial, Antifungal.*

Bazı 3,5-Disübstitüe-tetrahidro-2*H*-1,3,5-tiyadiazin-2-tiyon Türevlerinin Sentezi ve Antimikrobiyal Aktivitesi

*Uygun ve genel tek-kap yöntemle bir seri yeni 3,5-disübstitüe-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon (THTT) türevlerinin (**4a-g**) sentezi yapılmış ve bileşiklerin in vitro antibakteriyel ve antifungal etkileri mikrodilüsyon yöntemi kullanılarak, ampisilin ve flukonazol ile karşılaştırılarak incelenmiştir. 3-Fenil-5-(1-feniletil)-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon'un (**4a**) Staphylococcus aureus'a ve 3-fenil-5-hidroksi-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon'un (**4g**) Enterococcus faecalis'e karşı 4 ve 16 µg/mL MİK değerleriyle aktif olduğu bulunmuştur. 3-Fenil-5-(1-feniletil)-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon'un (**4a**) Candida krusei (MİK: 8 µg/mL) ve C. parapsilosis'e (MİK: 4 µg/mL) karşı antifungal aktivitesinin flukonazolden daha yüksek olduğu (MİK: 64 µg/mL ve 8 µg/mL) görülmüştür. 3-Fenil-5-(1-feniletil)-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon (**4a**) C. albicans'a karşı 4 µg/mL konsantrasyonda antifungal aktivite göstermiştir. 3-(1-Feniletil-5-[α-(izobutil)karboksimetil]-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon (**4b**) ve 3-benzil-5-karboksietil-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon'un (**4c**) C. krusei'ye karşı antifungal aktivitesinin flukonazole benzer olduğu bulunmuştur (MİK: 64 µg/mL).*

Anahtar kelimeler: *3,5-Disübstitüe-tetrahidro-2H-1,3,5-tiyadiazin-2-tiyon, Hidroksilamin, Ön ilaç, Antibakteriyel, Antifungal.*

[*]**Correspondence:** E-mail: mertan@hacettepe.edu.tr ; Tel: + 90 312 305 28 13; Fax: +90 312 311 47 77

INTRODUCTION

Many tetrahydro-1,3,5-thiadiazine-2-thiones (THTTs) and their derivatives are biologically important compounds since they exhibit antibacterial (1-6), antifungal (1, 2, 4-7), tuberculostatic (8, 9), antiprotozoan (10), antihelmintic (4), antifibrinolytic (11) and anticancer (12) activities. The biological activities of these compounds have been attributed to the isothiocyanates and dithiocarbamic acids which are formed by hydrolysis of the THTT ring (13, 14).

The prodrug approach has been particularly effective in decreasing pharmaceutical problems such as poor stability with amino-bearing drugs. THTT derivatives have been developed as a biolabile prodrug (15) in the design of drug delivery system for primary-amine-containing drugs due to their high lipid solubility and enzymatic and chemical rate of hydrolysis. In previous studies several aromatic and aliphatic primary amines (4, 16-18), amino acids (19-23), peptides (24) and primary-amine-containing drugs (6, 8, 25-29) such as 6-APA, ampicillin, amoxicillin, cephalexin, cefadroxil and isoniazid have been successfully attached to the THTT moiety to obtain prodrug which reveals higher lipophilicity and antibacterial and antifungal activity compared with the parent drug.

In continuation of our work in this area we aimed to synthesize some 3,5-disubstituted-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione derivatives to be used as prodrug and to evaluate their *in vitro* antibacterial and antifungal activities.

EXPERIMENTAL

Chemistry

All reagents were purchased in the higher quality available and were used without further purification. Melting points were determined on a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and are uncorrected. UV absorption spectra were measured on a Agilent 8453 UV-visible spectrophotometer. The IR spectra were recorded on a Bruker Vector 22 IR (Beaconsfield, UK) (KBr disc). ^{1}H-NMR spectra were taken in DMSO-d_6 using a Bruker AC 80 MHz FT NMR and Bruker Avance 400 MHz NMR (XWIN-NMR Software) spectrometers (Karlsruhe, Germany). Tetramethylsilane was used as the internal standard. ^{13}C-NMR spectra were measured on a Bruker Avance 400 MHz NMR (XWIN-NMR Software) using the same solvent and internal standard. All chemical shift values were recorded as δ (ppm). Mass spectra were taken on a 73DIP-1 Direct Insertion Probe using Agilent 5973-Network Mass Selective Detector (Ringoes, New Jersey, USA). The purity of the compounds was checked by thin-layer chromatography (silicagel, HF_{254}, type 60, 0,25 mm, E. Merck, Darmstadt, Germany). The elementary analyses of the compounds (C, H, N) were performed on a Leco CHNS 932 analyzer (Leco Co., St. Joseph, MI, USA) at the Scientific and Technical Research Council of Turkey, Instrumental Analyse Laboratory at Ankara, Turkey. The elementary analysis results were within 0.4% of theoretical values.

General procedure for the synthesis of 3,5-disubstituted-tetrahydro-2H-2-thiones

Carbon disulfide (0.6 mL, 0.01 mol) was added to a stirred mixture of the appropriate aryl- or aralkylamine (**1a-g**) (0.01 mol) and potassium hydroxide (20%, 2.8 mL, 0.01 mol); stirring was continued for 3 h at room temperature to form dithiocarbamic acid salt (**2a-g**). Then formaldehyde solution (37%, 1.63 mL, 0.022 mol) was added to the reaction medium and the stirring was continued for 1 h further. Oily residue formed in the reaction medium was removed by filtration. The clear filtrate was added dropwise to a stirred L-amino acid (0.01 mol) or primary amine (0.01 mol) suspension or hydroxylamine hydrochloride solution (0.695 g, 0.01

mol) in pH 7.8 phosphate buffer. The mixture was stirred for 4 h at room temperature and kept in the refrigerator overnight. The precipitate formed was removed by filtration. Then the filtrate was extracted three times with ether (20 mL). After removal of organic phase, the aqueous solution was cooled in an ice-bath and acidified with dilute hydrochloric acid (15%) to pH 2. The mixture was then stirred for 30 min at 0 °C and the precipitate was filtered under diminished pressure, washed with cold water, dried and recrystallized in a mixture of benzene and hexane (1:1).

3-Phenyl-5-(1-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4a**): Yield 42%, white powder, mp 178-180 °C. UV: λ_{max} (nm) (log ε) (MeOH): 203 (4.35), 245 (4.07), 292 (3.85). IR (KBr, cm^{-1}): ν 3026 (C-H aromatic), 2971 (C-H aliphatic), 1447 (C=S), 749, 686 (C-H monosubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 1.40 (d, 3H, -CH_3), 4.29 (q, 1H, -CH-), 4.63 (dd, J: 13.00 Hz, 2H, thiadiazine H-6), 4.69 (dd, J: 12.00 Hz, 2H, thiadiazine H-4), 7.20-7.50 (m, 10H, phenyl protons). MS (70 eV, EI): m/z (%) 256, 135, 105 (100), 91, 77. Anal. calcd. for $C_{17}H_{18}N_2S_2$ (314.46): C, 64.93; H, 5.77; N, 8.91; S, 20.39. Found: C, 64.90; H, 6.23; N, 8.78; S, 20.02.

3-(1-Phenylethyl-5-[α-(isobutyl)carboxymethyl]-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4b**): Yield 35%, white powder, mp 155-157 °C. UV: λ_{max} (nm) (log ε) (MeOH): 203 (4.19), 251 (3.84), 290 (3.95). IR (KBr, cm^{-1}): ν 3420 (O-H), 3050 (C-H aromatic), 2940, 2870 (C-H aliphatic), 1711 (C=O), 1459 (C=S), 778, 693 (C-H monosubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 0.70 (d, 6H, -CH(C<u>H</u>$_3$)$_2$), 0.85 (t, 2H, HOOC-CH-C<u>H</u>$_2$-CH-), 1.40 (d, 3H, C_6H_5-CH-C<u>H</u>$_3$), 1.45-1.60 (m, 1H, -CH_2-C<u>H</u>-(CH_3)$_2$), 3.30 (t, 1H, HOOC-C<u>H</u>-CH_2-), 3.80 (q, 1H, C_6H_5-C<u>H</u>-N-), 4.25 (dd, J: 12 Hz, 2H, thiadiazine H-6, 4.34 (dd, J: 16 Hz, 2H, thiadiazine H-4), 7.20-7.50 (m, 5H, phenyl protons). MS (70 eV, EI): m/z (%) 309, 264, 247, 201, 163, 128, 105 (100), 91, 77, 56. Anal. calcd. for $C_{17}H_{24}N_2O_2S_2$ (352.51): C, 57.92; H, 6.86; N, 7.95; S, 18.19. Found: C, 58.07; H, 7.00; N, 7.79; S, 16.33.

3-Benzyl-5-carboxyethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4c**): Yield 78%, mp 143-145 °C [mp 147-148 °C (19), mp 141-142 °C (20)].

3-(1-Phenylethyl)-5-hydroxy-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4d**): Yield 53%, white powder, mp 108-110 °C. UV: λ_{max} (nm) (log ε) (MeOH): 206 (4.15), 251 (3.70), 287 (3.85). IR (KBr, cm^{-1}): ν 3390 (O-H), 3023 (C-H aromatic), 2973, 2950, 2846 (C-H aliphatic), 1451 (C=S), 750, 690 (C-H monosubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 1.35 (d, 3H, -CH_3), 4.10 (q, 1H, -CH-), 4.45 (s, 2H, thiadiazine H-6), 4.55 (s, 2H, thiadiazine H-4), 7.10-7.30 (m, 5H, phenyl protons). ^{13}C-NMR (DMSO-d_6): δ 33.53 (CH_3), 53.25 (CH), 57.77 (C-6), 70.57 (C-4), 126.57 (phenyl, C-1), 128.77 (phenyl, C-2, C-6), 129.16 (phenyl, C-3, C-5), 138.70 (phenyl, C-1), 191.00 (C=S). MS (70 eV, EI): m/z (%) 342 (49), 253, 238, 197, 179, 163, 146, 132, 104 (100), 91, 77, 65, 51. Anal. calcd. for $C_{11}H_{14}N_2OS_2$ (254.36): C, 51.94; H, 5.55; N, 11.01; S, 25.21. Found: C, 52.07; H, 6.03; N, 10.78; S, 24.82.

3-Benzyl-5-hydroxy-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4e**): Yield 58%, white needle crystals, mp 101-103 °C. UV: λ_{max} (nm) (log ε) (MeOH): 203 (4.24), 249 (3.77), 290 (3.91). IR (KBr, cm^{-1}): ν 3021 (C-H aromatic), 2946, 2903, 2842 (C-H aliphatic), 1488 (C=S), 740, 693 (C-H monosubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 3.75 (s, 2H, C_6H_5-C<u>H</u>$_2$-), 4.40 (s, 2H, thiadiazine H-6), 4.50 (s, 2H, thiadiazine H-4), 7.00-7.50 (m, 5H, phenyl protons). MS (70 eV, EI): m/z (%) 240 (M^+), 224, 165, 149, 133, 118, 91 (100), 76, 65, 56. Anal. calcd. for $C_{10}H_{12}N_2OS_2$ (240.34): C, 49.98; H, 5.03; N, 11.66; S, 26.68. Found: C, 50.30; H, 4.93; N, 11.45; S, 26.40.

3-(4-Carboxyphenyl)-5-hydroxy-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4f**): Yield 43%, pale yellow powder, mp 225-227 °C. UV: λ_{max} (nm) (log ε) (MeOH): 202 (4.19), 219 (4.14), 290 (4.01). IR (KBr, cm^{-1}): ν 3400 (O-H), 3059 (C-H aromatic), 2979, 2881, 2832 (C-H aliphatic), 1694 (C=O), 1420 (C=S), 840, 773 (C-H p-disubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 3.50 (broad s, 1H, N-OH, 5.35 (s, 2H, thiadiazine H-6), 5.45 (s, 2H, thiadiazine H-4), 7.30-8.10 (m, 4H, p-substituted phenyl protons), 13.00 (broad singlet, 1H, -COOH). ^{13}C-NMR (DMSO-d_6): δ 54.76 (C-6), 69.88 (C-4), 116.90 (phenyl, C-2, C-6), 124.12 (phenyl, C-4), 131.54 (phenyl, C-3, C-5), 148.65 (phenyl, C-1), 167.35 (C=O), 194.09 (C=S). MS (70 eV, EI): m/z (%) 179, 149 (100), 137, 120, 92, 76, 65. Anal. calcd. for $C_{10}H_{10}N_2O_3S_2$ (270.32): C, 44.43; H, 3.73; N, 10.36; S, 23.72. Found: C, 43.86; H, 4.12; N, 10.44; S, 23.87.

3-Phenyl-5-hydroxy-tetrahydro-2H-1,3,5-thiadiazine-2-thione (**4g**): Yield 50%, yellow powder, mp 162-164 °C. UV: λ_{max} (nm) (log ε) (MeOH): 203 (4.35), 245 (4.06), 292 (3.83). IR (KBr, cm^{-1}): ν 3328 (O-H), 3046 (C-H aromatic), 2920, 2872 (C-H aliphatic), 1497 (C=S), 751, 687 (C-H monosubstituted benzene). ^{1}H-NMR (DMSO-d_6): δ 5.25 (s, 2H, thiadiazine H-6), 5.45 (s, 2H, thiadiazine H-4), 7.00-7.50 (m, 5H, phenyl protons). ^{13}C-NMR (DMSO-d_6): δ 55.42 (C-6), 69.86 (C-4), 117.71 (phenyl, C-2, C-6), 122.26 (phenyl, C-4), 127.48 (phenyl, C-3, C-5), 144.57 (phenyl, C-1), 193.86 (C=S). MS (70 eV, EI): m/z (%) 151, 135, 105 (100), 91, 77. Anal. calcd. for $C_9H_{10}N_2OS_2$ (226.30): C, 47.77; H, 4.45; N, 12.38; S, 28.33. Found: C, 47.96; H, 4.56; N, 12.73; S, 28.46.

Microbiology

MICs values were determined by microdilution broth method following the procedures recommended by the NCCLS (30, 31). Ampicillin and fluconazole were used as the reference drugs for bacteria and fungi, respectively. The compounds were tested against two Gram-positive (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and three *Candida* sp. (*Candida albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) by using microdilution broth method. MIC values of the compounds are presented in Table 1. Reference drugs were dissolved in sterile distilled water. The stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO). The dilutions in the test medium were prepared at the required concentration of 1024-0.5 μg/mL and for reference drugs 128-0.125 μg/mL. The final inoculum densities were 5 x 10^5 cfu/mL for bacteria and 0.5-2.5 x 10^3 cfu/mL for fungi. MIC was defined as the lowest concentration of the compound that inhibited visible growth of microorganisms. It was established that dilution of DMSO lacked antimicrobial activity against any of the test microorganisms.

Antibacterial activity assay

The cultures were grown on Mueller-Hinton Agar (MHA) (BBL, MD, USA) for all bacteria after 18-24 h of incubation at 35 °C. Before the assay, all of the bacteria were grown in Mueller-Hinton Broth (MHB) for 2-6 hours. Then the bacterial suspensions were adjusted to 0.5 Mc Farland turbidity (1 x 10^8 cfu/mL). The microtiter plates were incubated at 35 °C and inspected visually after 18-24 h for bacteria. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

Antifungal activity assay

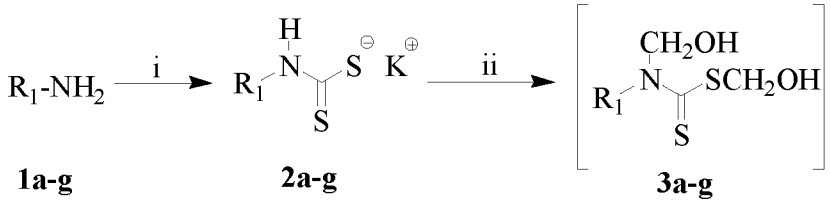
All fungi were cultivated in Sabouraud Dextrose Agar (Merck). RPMI-1640 medium (ICN-Flow, Aurora, OH, USA) with L-glutamine, buffered with 3-(*N*-morpholino)propanesulphonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH-USA) at pH=7.4 is used as the test medium. The microtiter plates were incubated at 35 °C and evaluated visually after 48 h. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

RESULTS AND DISCUSSION

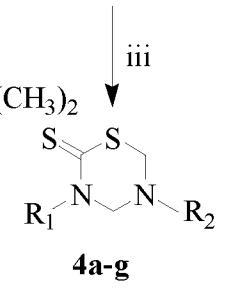
Chemistry

Several methods have been reported for the synthesis of THTTs such as the use of isothiocyanate (32) and solid phase organic synthesis (SPOS) methodology (33) but the most convenient method proceeds via a dithiocarbamate salt intermediary (18-20, 26-29, 33, 34). This simple and direct method for the synthesis of 3,5-symmetrically substituted THTT derivatives involves reaction of 2 mol primary amines with 1 mol carbon disulfide to give the amine salt of dithiocarbamic acid which was not isolated, then cyclocondensation with 2 mol formaldehyde (1, 4, 17, 18, 34, 35). For instance, the reaction of 2 mol benzylamine with 1 mol carbon disulfide and 2 mol formaldehyde gives 3,5-dibenzyl-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (18). The described approach has been also applied to the synthesis of tetrahydro-1,3,5-thiadiazine-2-thiones having different substituents at the N^3 and N^5 atoms by the reaction of primary amine and alkali hydroxide with carbon disulfide to give dithiocarbamic acid salt followed by cyclisation with "additional amine molecule" and 2 mol formaldehyde (3, 5, 17). This method is very flexible and gives possibility to prepare a large number of THTTs bearing various substituents in tetrahydro-1,3,5-thiadiazine-2-thione ring.

In order to synthesize the target compounds (**4a-g**) selected primary amines, including aniline, (+)-1-phenylethylamine, benzylamine and p-aminobenzoic acid were reacted with carbon disulfide and potassium hydroxide to form their corresponding potassium dithiocarbamate derivatives (which were not isolated) (**2a-g**). Addition of formaldehyde and (+)-1-phenylethylamine (**4a**), appropriate L-amino acids (leucine and alanine) (**4b-c**) or hydroxylamine (**4d-g**) to the potassium dithiocarbamates resulted in the desired 3,5-disubstituted-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione derivatives in fair to good yields (Scheme 1).



4a: R_1= C_6H_5; R_2= $CH(CH_3)C_6H_5$
4b: R_1= $CH(CH_3)C_6H_5$; R_2= $CH(COOH)CH_2CH(CH_3)_2$
4c: R_1= $CH_2C_6H_5$; R_2=$CH(CH_3)COOH$
4d: R_1= $CH(CH_3)C_6H_5$; R_2= OH
4e: R_1= $CH_2C_6H_5$; R_2= OH
4f: R_1= $C_6H_4COOH(p)$; R_2= OH
4g: R_1= C_6H_5; R_2= OH



Scheme 1: Synthesis of compounds **4a-g**. Reagents and conditions: (i) KOH (20%), CS_2, rt; (ii) HCHO; (iii) H_2N-R_2 [(+)-1-phenylethylamine, various L-amino acids or H_2N-OH HCl].

Although the synthesis and antifungal activity of compound **4c** have been previously reported (19, 20), it is included for the examination of its antimicrobial activity.

The structures of the compounds were established on the basis of spectroscopic data and elementary analysis. All UV spectra have three absorption maxima in the 202-206, 219-251 and 287-292 nm range belong to 3,5-disubstituted-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione ring (35, 36). The IR spectra of the compounds showed absorption bands at the range 3059-3021 cm^{-1} (aromatic C-H stretching), 2979-2832 cm^{-1} (aliphatic C-H stretching) and 1497-1420 cm^{-1} (C=S stretching). Compounds **4b**, **4c** and **4f** carrying COOH group at the N^5 or N^3 position of the tetrahydrothiadiazine-2-thione structure displayed the characteristic stretching absorption of the carboxylic acid in the range 3420-3400 cm^{-1} (O-H) and 1711-1692 cm^{-1} (C=O). In the IR spectra of N^5-hydroxy substituted derivatives (**4d-g**), O-H stretching bands observed at the range 3400-3328 cm^{-1}, except compound **4e**.

The ^{1}H-NMR spectra of the compounds **4a** and **4b** showed two double doublets corresponding to the H-6 and H-4 protons of the thiazolidine ring, which could be explained by magnetic anisotropy and semi-chair conformation of the thiadiazine ring (19, 37). These protons yielded AB systems because of their diastereotopicity due to the chiral substituent on N^5 and were observed at δ 4.63, 4.25 ppm (H-6) and δ 4.69, 4.34 ppm (H-4), respectively (38). However in the case of N^5-hydroxy substituted derivatives (**4d-g**) the expected diastereotopic methylene protons of the thiazolidine ring could not be observed and these protons appeared as two separate singlets at around δ 4.40-5.35 and 4.50-5.45 ppm, respectively. The exchangeable N^5-hydroxy protons were not observed in the ^{1}H-NMR spectra, except **4f**. In compound **4f** the signals of N^5-OH and –COOH protons were observed as broad singlets at δ 3.50 and 13.00 ppm, respectively. All the other protons resonated in the expected regions and integral values.

The ^{13}C-NMR data of the compounds **4d**, **4f** and **4g** displayed characteristic signals for C=S, C-4 and C-6 appeared at about δ 191.00-193.86, 69.86-70.57 and 54.76-57.77 ppm, respectively (38).

In the EI-Mass spectra, only compound **4e** showed very weak molecular ion peak at m/z 240. Under EI conditions compounds **4a** and **4b** cleaved with formation of the stable 1-phenylethyl cation (m/z 105) as the base peak (39). The transposition product ion 3,5-bis(1-phenylethyl)-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione originated from 1-phenylethyl cation, was formed at m/z 342 in high relative percentage in compound **4d** (37). Loss of 4-carboxyphenyl group produced the base peak at m/z 149 in compound **4f**.

Antimicrobial activity

Table 1 indicates that compound **4a** was more effective against *S. aureus* (MIC: 4 μg/mL) than all the other derivatives. Compounds **4b-c** and **4e-f** displayed similar antibacterial activity against *S. aureus* with MIC of 32 and 64 μg/mL, respectively. Compound **4g,** 3-phenyl-5-hydroxy-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione, showed activity against *E. faecalis* at 16 μg/mL concentration while ampicillin was active at 8 μg/mL. None of the compounds proved to be effective against Gram (-) bacteria.

Table 1. Antibacterial and antifungal activity of title compounds **4a-g** (MIC in μg/mL).

	Antibacterial activity				Antifungal activity		
Compounds	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. krusei*	*C. parapsilosis*
4a	4	128	256	512	4	8	4
4b	32	128	256	256	64	64	64
4c	32	64	128	256	64	64	64
4d	>512	512	>512	>512	256	>512	512
4e	64	256	>512	>512	128	256	128
4f	64	256	512	512	64	128	32
4g	>512	16	>512	512	128	512	256
Ampicillin	1	8	2	-	-	-	-
Fluconazole	-	-	-	-	1	64	8

All compounds, except **4d**, **4e** and **4g** showed antifungal activity against *Candida* species. Especially introduction of N^3-phenyl group into the ring system gives a good profile of antifungal activity. The antifungal activity of compound **4a**, 3-phenyl-5-(1-phenylethyl)-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione, against *C. krusei* and *C. parapsilosis* appeared greater than that of fluconazole (MIC: 64 μg/mL and 8 μg/mL) with MIC of 8 and 4 μg/mL, respectively. Compound **4a** also displayed significant antifungal activity against *C. albicans* at 4 μg/mL concentration while fluconazole was active at 1 μg/mL. Antifungal activity of compounds **4b** and **4c** against *C. krusei* were found to be the same as fluconazole (MIC: 64 μg/mL). Among the N^5-hydroxy substituted derivatives, only one compound with 4-carboxyphenyl substituent at N^3 (**4f**) was found to be potent against *C. albicans* and *C. parapsilosis* with MIC of 64 and 32 μg/mL, respectively.

CONCLUSION

In this study, the synthesized compounds have been designed according to the fact that in thiadiazine-2-thiones N^3 and N^5 substitutions with hydrophobic and hydrophilic groups, respectively, leads better activity and low toxicity to the host (3). The antibacterial and antifungal activity of newly synthesized THTT derivatives are both significantly reduced by the introduction of a polar hydroxyl group into the N^5 position of the ring. Hydrolysis of these compounds generates isothiocyanates which could be further formed corresponding hydroxythiourea derivatives. Therefore evaluation of the chemical and enzymatic degradation kinetics of N^5-hydroxy substituted THTT derivatives (**4d-g**) is being planned in our future studies. Since hydroxythioureas themselves are associated with antimicrobial, antituberculostatic and sitostatic activities (12) further evaluation of compounds **4d-g** could be an advantage in developing new drugs. Preparation of N^5-alkoxy- and N^5-acyloxy- derivatives to increase the lipophilicity of the molecules and testing of their antimicrobial properties are currently being carried out in our laboratory.

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