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SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF 2-THIONE-3-SUBSTITUTED-5-(4-CARBOXYCYCLOHEXYL-METHYL)-TETRAHYDRO-2H-1,3,5-THIADIAZINE DERIVATIVES

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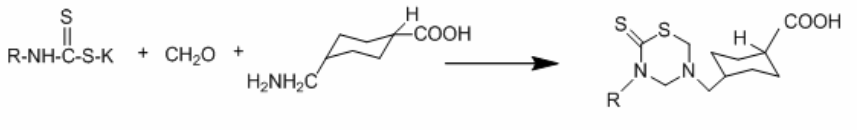
In this study, 3-substituted-5-(4-carboxycyclohexyl-methyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione which have phenylethyl (1), octyl (2), cyclopropyl (3), 4-methoxybenzyl (4), phenyl (5) groups in position 3 have been synthesized and examined for antimicrobial activities. Their structures were elucidated by spectral method. Antibacterial activities of these compounds against Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212), gram-negative bacteria (*Escherichia coli* ATCC 2592, *Pseudomonas aeruginosa* ATCC 27853) and yeast-like fungi (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018) were investigated by the micro-dilution method and compared with the activity of sulbentine, ciprofloxacin and flucanozole. By this way their minimal inhibitory concentration (MIC) values were determinated. Compounds 1-4 exhibited almost equally potent activity against *Staphylococcus aureus* ATCC 29213 (MIC: 31.2 µg/mL). Compounds 1,3,4 showed similar antibacterial activity against *Enterococcus faecalis* ATCC 29212) (MIC: 62.5 µg/mL). None of the compounds exhibited activity against Gram-negative bacteria. On the other hand, all compounds had potent antifungal activities against the yeast utilized. Compounds 1-4 displayed significant antifungal activity against *Candida parapsilosis* ATCC 90018 at 7.8 µg/mL concentration while sulbentine was active at 125 µg/mL. Among the synthesized compounds, 3- cyclopropyl-5-(4-carboxycyclohexyl-methyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione(3) seems to be the most effective compound with antibacterial and antifungal activity.



INTRODUCTION

It is known that substituted tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives have several biological activities such as antibacterial,[1-10] antifungal,[11-17] antiprotozoal,[18,19] antifibrinolytic,[20,21] anticancer,[22,23] antituberculostatic.[24,25] Derivatives of tetrahydro-2H-1,3,5-thiadiazine-2-thione are in the market for dermatomycosis treatment and used against various soil fungi and some nematodes as a phyto-protective agent.[26] Isothiocyanates cannot be considered suitable chemotherapeutics in human medicine because of their undesired pharmaceutical and physicochemical properties that they are used as prodrugs.[27] For this reason, we aimed to prepare a series of compounds such as tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives, as prodrugs, which converted to isothiocyanates in an aqueous solution.[28]

Sulbentine, which has a broad spectrum effect and used especially in dermatomycoses treatment is a drug used in the treatment of various forms.

In this study, 3-substituted-5-(4-carboxycyclohexyl-methyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione which have phenylethyl (1), octyl (2), cyclopropyl (3), 4-methoxybenzyl (4), phenyl (5) groups in position 3 have been synthesized and examined for antimicrobial activities.

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RESULTS

Chemical synthesis

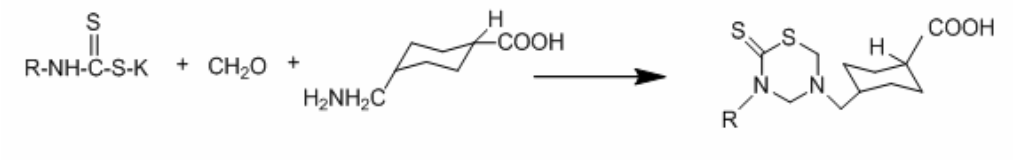
Compounds 1-5 were synthesized by the reaction of dithiocarbamic acid salts prepared from primary amines, with formaldehyde and tranexamic acid (Scheme 1).

Reaction formation has two different mechanisms in the literature (Scheme 2).[16,29]

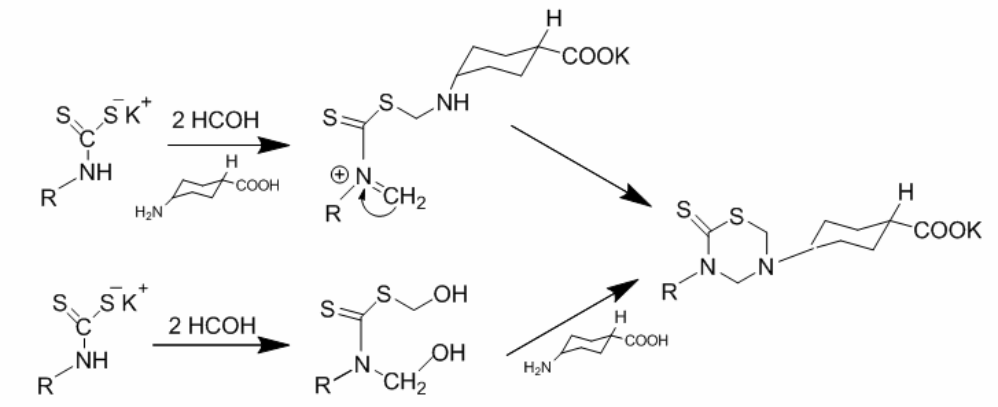
The synthesis results and spectral data of 3-substituted-5-(4-carboxycyclohexyl-methyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives are given in Table 1.

The IR spectra of the compounds showed absorption bands at range 3026 cm^{-1} (aromatic C-H stretching), 2940-2827 cm^{-1} (aliphatic C-H stretching) and 1460-1515 cm^{-1} (C=S stretching). All compounds carrying COOH group displayed the characteristic stretching absorption of the carboxylic acid in the range 3300-2900 cm^{-1} (O-H) and 1705-1680 cm^{-1} (C=O).

The ^{1}H-NMR spectral data of the compounds were found to be in agreement with the data expected and reported in the literature.[30] It was indicated that H4 and H6 protons of the 1,3,5-thiadiazine ring could be seen together as a singlet at about 4.40-4.7 ppm or could be seen separately. H6 protons occured at about 4.60–5.10 ppm as a singlet and H4 protons at about 4.50–5.00 ppm as double singlet.[30]



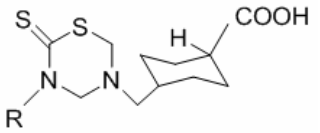
Scheme 1– General synthesis of the compounds.



Scheme 2 – Mechanism of reaction.

Table 1

Melting points, yields and spectral data of compounds



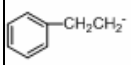
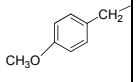
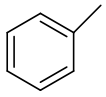
Comp.	R	Yield (%)	M.p. (^{0}C)	Molecular formula (M. W)	IR (KBr)cm^{-1}	^{1}H NMR(DMSO-d_6) ppm (δ)
1		57	170-172	$C_{19}H_{27}N_2O_2S_2$ MS(ESI^+) Calculated:379.1514 Found: 379.1506	3300-2900 (O-H), 3026 (C-H aromatic), 2940,2853 (C-H aliphatic), 1684 (C=O), 1494 (C=S)	7.33-7.2 (m,5H, phenyl protons), 4.45 (s, 2H, thiadiazine H4), 4.41 (s, 2H, thiadiazine H6), 4.1 (t, 2H, - $\underline{CH_2}$-CH_2-phenyl), 2.9 ((t, 2H, - CH_2-$\underline{CH_2}$-phenyl), 2.4 (d, 2H, CH_2-thiadiazine N5), 0.9-2.1 (m, 10H, cyclohexane)

Table 1(continued)

2	C_8H_{17}-	47	149	$C_{19}H_{35}N_2O_2S_2$ MS (ESI^+) Calculated: 387.2140 Found: 387.2141	3300-2900 (O-H), 3026 (C-H aromatic), 2933,2851 (C-H aliphatic), 1705 (C=O), 1505 (C=S)	12 (s, 1H,-COOH), 4.46 (s, 2H, thiadiazine H4), 4.43 (s, 2H, thiadiazine H6), 3.9 (t, 2H, - $\underline{CH_2}$-C_7H_{15}) , 2.4 (d, 2H, CH_2-thiadiazine N5), 0.8-2.1 (m, 25H, octil protons, cyclohexane)
3		42	141	$C_{14}H_{22}N_2O_2S_2$ MS (ESI^+) Calculated: 315.1201 Found: 315.1210	3300-2900 (O-H), 3026 (C-H aromatic), 2933,2850 (C-H aliphatic), 1690(C=O), 1453 (C=S)	4.13 (s, 4H, thiadiazine H4 and H6), 3.1 (t, 1H, - $\underline{CH}$, cyclopropyl), 2.4 (d, 2H, CH_2-thiadiazine N5), 0.8-2.1 (m, 14H, cyclopropyl protons, cyclohexane)
4		53	215	$C_{19}H_{27}N_2O_2S_2$ MS (ESI^+) Calculated: 395.1554 Found: 395.1463	3300-2900 (O-H), 3006 (C-H aromatic), 2929,2827 (C-H aliphatic), 1703 (C=O), 1514 (C=S)	7.4 (d,2H, phenyl protons), 6.9 (d,2H, phenyl protons), 5.2 (s, 2H, 4-methoxy benzyl), 4.48 (s, 2H, thiadiazine H4), 4.33 (s, 2H, thiadiazine H6), 3.76 (s, 3H, methoxy) , 2.2 (d, 2H, CH_2-thiadiazine N5), 0.9-2.1 (m, 10H, cyclohexane)
5		44	170	$C_{17}H_{23}N_2O_2S_2$ MS (ESI^+) Calculated: 351.1201 Found: 351.1201	3300-2900 (O-H), 3026 (C-H aromatic), 2934,2849 (C-H aliphatic), 1705 (C=O), 1460 (C=S)	12 (s, 1H,-COOH), 7.5-7.2 (m,5H, phenyl protons), 4.65 (s, 2H, thiadiazine H4), 4.63 (s, 2H, thiadiazine H6), 2.7 (d, 2H, CH_2-thiadiazine N5), 0.9-2.1 (m, 10H, cyclohexane)

Antimicrobial activity

The synthesis of title compounds and their antimicobacterial activity were described. The results of antibacterial and antifungal activity results are shown in Tables 2-3 and it was found that antimycobacterial activity of the compound 3 was the most prominent when compared to sulbentine.

The aim of this study was to prepare some new compounds to be used as prodrug, in which amino groups of tranexamic acid are members of tetrahydro-2H-1,3,5-thiadiazine-2-thione ring. It was also aimed to investigate their *in vitro* antibacterial and antifungal activities compared to sulbentine, ciprofloxacin and flucanozole. The antibacterial activity of the tested compounds was investigated *in vitro* against *E.coli* ATCC 2592, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and the antifungal activity of the tested compounds was investigated *in vitro* against *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018. The broth micro-dilution method was used for determination of antibacterial and antifungal activity after determination of minimal inhibitory concentrations (MIC).

All compounds had potent antifungal activities against the utilized fungi. Compounds 1-4 displayed significant antifungal activity against *Candida parapsilosis* ATCC 90018 at 7.8 µg/mL concentration while sulbentine was active at 125 µg/mL. Moreover, compound 5 was found to be potent against *Candida parapsilosis* ATCC 90018 with MIC of 31.25 µg/mL. Compounds 2,3,4 exhibited similar activity against *Candida albicans* ATCC 10231 (MIC: 15.62 µg/mL). Among the synthesized compounds, besides, compound 1 showed antifungal activity similar to sulbentine whereas other compound 5 had less activity.

Compounds 1-4 exhibited almost equally potent activity against *Staphylococcus aureus* ATCC 29213 (MIC: 31.2 µg/mL). Compounds 1,3,4 showed similar antibacterial activity against *Enterococcus faecalis* ATCC 29212) (MIC: 62.5 µg/mL). None of the compounds exhibited activity against Gram-negative bacteria.

Table 2

Antibacterial activities of chemical derivatives (μg / mL)

Compunds	*E.coli* ATCC 25922	*Pseudomonas. aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212
1	125	250	31. 25	62. 5
2	125	500	31. 25	250
3	250	250	31. 25	62. 5
4	250	500	31. 25	62. 5
5	250	500	125	250
Sulbentin	500	500	250	500
Ciprofloxacin	0.04	0.64	0.32	0. 64
Fluconazole	-	-	-	-

Table 3

Antifungal activities of chemical derivatives (μg / mL)

Compunds	*Candida albicans* ATCC 10231	*Candida parapsilosis* ATCC 90018
1	31. 25	7. 8
2	15. 62	7. 8
3	15. 62	7. 8
4	15. 62	7. 8
5	62. 5	31. 25
Sulbentin	31. 25	125
Ciprofloxacin	-	-
Fluconazole	1.28	0. 64

EXPERIMENTAL

Materials and methods

Chemistry

The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany). Tranexamic acid (CAS 1197-18-8) was obtained from Fako (Fako Pharmaceutical Firm, İstanbul, Turkey). Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the given values are uncorrected. The IR spectra of the compounds were recorded on a Bruker Vector 22 IR spectrophotometer (Bruker Analytische Messtechnik, Karlrure, Germany). The ^{1}H-NMR of the compounds spectra were recorded on a Bruker 400 MHz-NMR Spectrometer (Rheinstetten, Karlrure, Germany) using tetramethylsilane as an internal standard. All the chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-fligth instrument) operating in either ESI (+) methods, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA,USA).

Methods

3-substituted-5-(4-carboxycyclohexyl-methyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione

Carbon disulfide (0.6 mL. 0.01 mol) was added to a stirred mixture of primary amine (0.01 mol) potassium hydroxide (20 % 2.8 mL. 0.01 mol). The mixture was stirred for 3 h at room temperature. Then formaldehyde solution (37 % 1.63 mL. 0.022 mol) was added to the reaction mixture in which the dithiocarbamic acid salt was formed. After stirring for 30 min. oily residue formed in the reaction medium was removed by filtration. The clear filtrate obtained was added dropwise to a stirred tranexamic acid suspension (1.57g, 0.01 mol) in pH 7.8 phosphate buffer. The mixture was stirred for 4h and then left overnight in the refrigerator. The mixture was then extracted with ether (20 mL) for 3 times. Organic layer was removed. The aqueous solution was cooled in an ice-bath and acidified with dilute hydrochloric acid to pH 2. The solution was then stirred for 30 min at 0^{o} C and the precipitate formed was filtered, washed with cold water and crystallized with ethanol and dried. The synthetic route has been shown in Scheme 1.

Sulbentine which was used as a standard substance was synthesized by us and the melting point was consistent with literature.

Synthesis of Sulbentine

1.08 mL benzylamine and 2.6cc %28 formaldehyde were poured in 5 cc ethanol and then stirred for 30 min at room temperature. Then 0.4 cc CS_2 was added to the reaction mixture and the mixture was stirred for 10 minutes and heated for 10 minutes. 3,5-dibenzil tetrahidro-1,3,5-thiadiazine was obtained by reaction.

Melting point: 93^{0}C (102-103 ^{0}C) [31]

$C_{17}H_{19}N_2S$ MS (ESI^{+}) Calculated: 315,0990 Found: 315,0984

Biological activities

Materials

Mueller Hinton Agar (MHA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), Sabouraud Liquid Medium Agar (SLM) (Merck),

RPMI-1640 medium with L-glutamine (Sigma), 3- [N-morpholino]-propansulfonic acid (MOPS) (Sigma), 96 well microplates (Falcon), transfer pipette (Biohit), ciprofloxacin (Sigma), fluconazole (Nobel), dimethylsulphoxide (DMSO) (Riedel de Haen).

Microorganisms

Two gram-negative *(Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922) and two gram-positive (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212) bacteria were used as quality control strains.[32] For determining anti-yeast activities of the compounds, the following reference strains were tested: *C. albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018.[33]

Methods

Standard powders of ciprofloxacin and fluconazole were obtained from the manufacturers. Stock solutions were dissolved in distilled water (ciprofloxacin and fluconazole).

All bacterial isolates were subcultured in MHA plates and incubated over night at 37 ^{0}C and all Candida isolates were subcultured in SDA plates at 35 ^{0}C for 24-48 h. The microorganisms were passaged at least twice to ensure purity and viability.

The solution of the newly synthesized compounds and standard drugs were prepared at 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, 1.95, 0.975 µg/mL concentrations, at 10.24, 5.12, 2.56, 1.28, 0.64, 0.32, 0.16 0.08 0.04, 0.02 µg/mL concentrations in the wells of microplates by diluting in MHB, respectively.

Bacterial susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) M100-S16.[32] The bacterial suspensions used for inoculation were prepared at 10^5 cfu/mL by diluting fresh cultures at MacFarland 0.5 density (10^7 cfu/mL). Suspensions of the bacteria at 10^5 cfu/mL concentration were inoculated to the twofold diluted solution of the compounds. There were 10^4 cfu/mL bacteria in the wells after inoculations. MHB was used for diluting the bacterial suspension and for twofold dilution of the compound. DMSO, pure microorganisms and pure media were used as control wells. A 10 µl bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37 ^{0}C in a humid chamber and MIC endpoints were read after 24 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported.

All Candida isolates were subcultured in SDA plates, and incubated at 35 ^{0}C for 24-48 h prior to antifungal susceptibility testing, and passaged at least twice to ensure purity and viability. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered pH 7 with MOPS and culture suspensions were prepared through the guideline of CLSI M27-A.[33] The yeast suspensions used for inoculation were prepared at 10^4 cfu/ml by diluting fresh cultures at MacFarland 0.5 density (10^6 cfu/mL). Suspensions of the yeast at 10^4 cfu/mL concentration were inoculated to the twofold diluted solution of the compounds. There were 10^3 cfu/mL yeast in the wells after inoculations. A 10 µL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 ^{0}C in a humid chamber and MIC endpoints were read after 48 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported in Table 2.

CONCLUSIONS

Compared with the sulbentine which can be easily found in the market because of its antifungal activity, 3-cyclo-5-(4-carboxycyclohexylmethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione (compound 3) is more active and we think that our compund which is the most effective compound as antibacterial and antifungal activity can be used as broad spectrum antifungal in the future.

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