Research Article

Synthesis and Biological Activity of Some Thiazole Derivatives with Dithiocarbamate side chaine

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ABSTRACT

In this study we aimed to synthesize new 2-((5-substituted-4-methylthiazole-2-yl)amino)-2-oxoethyl-4substitutedpiperazine-1-carbodithionate derivatives and studying their antibacterial, antifungal effects as well as ACHE inhibitory evulation. A set of fourteen new compounds of 2-((5-substituted-4-methylthiazole-2-yl)amino)-2-oxoethyl-4substitutedpiperazine-1-carbodithionate derivatives were synthesized by reacting 2-chloro-N-(5-substituted-4-methylthiazole-2-yl)acetamide derivatives with sodium salts of appropriate N-substituted piperazine dithiocarbamate acids in acetone. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR and MS spectral data. These compounds were screened for their *in vitro* antimicrobial activity. Among these derivatives; eight compounds were found to be the most promising antimicrobial agents against Pseudomonas aeruginosa. These compounds and streptomyicine exhibited the same level of antibacterial activity with a MIC value of value of 125 µg/mL, whereas other derivatives showed their antibacterial activity against *Pseudomonas aeruginosa* with a MIC value of 250 µg/mL. All compounds exhibited their inhibitory activity against *Fusarium solani* and *Candida krusei* with a MIC value of 250 µg/mL when compared with ketoconazole (MIC= 62.5 µg/mL). ACHE inhibitory activities of the synthesized derivatives were tested against Donepezil for their ability to inhibit acetylcholinesterase (ACHE) using a modification of Ellman's spectrophotometric method. However no significant inhibitory activity was observed.

Keywords: ACHE, Antibacterial, Antifungal, Dithiocarbamate, Thiazole.

INTRODUCTION

It is known that; compounds carrying dithiocarbamate group have different pharmacological activities, such as antimicrobial[1], antifungal[2], antiproliferative[3] pesticide[4] and anticholinesterase.[5] The dithiocarbomate residue is associated with fungicides, this group have been widely used for protecting crops against many fungal diseases. They are characterized by their ethylenebisdithiocarbamate residue and different metals in core structure.[2] In the last decades the chemistry of dithiocarbamate residue bearing heterocyclic compounds received great attention. A number of compounds have been synthesized and diverse antibacterial and antifungal activities have been investigated.[6-9] Thiocarbonyl aromatic compounds was greatly affected by the lipophilicity, that is obtained by thiocarbonyl moiety, especially the calculated log P value and the balance between hydrophilic substituent and hydrophobic substituent on the aromatic compounds. Thiazole nucleus is also found as a part of various natural products and biologically active compounds, is selected as the complementary part of the target compounds of this work, Thiazole also process a wide variety of biologically activites.[10] Many studies have emerged on thiazole as potent antimicrobial agents[11-16] and anticholinesterase activity.[17-20] Thus, with the selection of the thiazole ring as the complementary part. Anticholinesterase activity is of importance hence inhibition of acethylcholinestrease(ACHE) is a way of treatment of Alzhemier's disease(AD), seniledementia, myasthenia gravis and Parkinson's disease.[21] In this study, in order to combine the dithiocarbamate and thiazole residues and their above mentioned potential biological activities in a single structure some2-((5-substituted-4-methylthiazole-2-yl)amino)-2-oxoethyl-4-substituted piperazine-1-carbodithioates were synthesized to investigate their antibacterial, antifungal, and acetylcholinesterase inhibitory effects.

EXPERIMENTAL

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO). All melting points (m.p.) were determined by Electrothermal 9100 digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H-NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-d_6 using TMS as internal standard; M+1 peaks were determined by AB Sciex-3200 Q-TRAP LC/MS/MS system (AB Applied Biosystems Co., MA, USA).

General procedure for synthesis of the compound

2-Chloro-*N*-[5-substituted-4-methylthiazole-2yl] acetamide derivatives

A solution of 2-amino-5-substituted-4-methylthiazole (1) (0,01mole) and triethylamine (0.02 mol) was prepared by stirring in dry THF (15 mL) at room temperature. After cooling the mixture in an ice bath, Chloroacetyl chloride (0.01 mol) was added drop-wise over 15 minutes to a magnetically stirred. Before evaporating the solvent under reduced pressure. The reaction mixture was further stirred for 1 hour at room temperature. The precipitate formed was crystallized from ethanol.[22,6]

Sodium salts of N, N-disubstituted dithiocarbamic acids

Sodium hydroxide (10 mmol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (10 mmol) the mixture was cooled in an ice bath and carbon disulfide (100 mmol) was added drop wise with stirring. The reaction mixture was stirred for 1 h at room temperature. The products were afforded by filtration and washed with diethyl ether.[23]

2-((5-substituted-4-methylthiazol-2-yl) amino)-2-oxoethyl 4-substituted piperazine-1-carbodithioate derivatives

A suitable 2-Chloro-*N*-[5-substituted-4-methylthiazole-2yl]acetamide derivatives was stirred with appropriate sodium salts of dithiocarbamic acids (0.0011 mol) in acetone for 3 h. After evaporating the solvent under reduced pressure, the precipitated product was washed with water and the residue was crystallized from ethanol.[6,9]

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-phenylpiperazine-1-carbodithioate (5a)

Yield: %84. M.p. 187-189 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3266 (amide N-H), 1674 (amide C=O), 1540-1650 (C=C , C=N) 1214(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3)2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,19(twobs, 4H, piperazine C2.6-H),4.32(s,2H,CO$\underline{CH_2}$),7.34-7.38(m,3H,Ar-H), 7.47(d, 2H, Ar-H) 12.22 (s, 1H, N-H)MS (ES+) : m/z 407.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-(4-fluorophenyl)piperazine-1-carbodithioate(5b)

Yield: %67. M.p. 186-188 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3276 (amide N-H), 1680 (amide C=O), 1532-1640 (C=C , C=N) 1211(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3)2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,19(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7,80(d,2H,Ar-H),7.93(d,2H,Ar-H) 12,18 (s, 1H, N-H)MS (ES+) : m/z 425.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate(5c)

Yield: %84. M.p. 187-189 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3266 (amide N-H), 1678 (amide C=O), 1544-1652 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3)2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H), 4.32(s,2H,CO$\underline{CH_2}$), 7.89(d,2H,Ar-H),8.10 (d,2H,Ar-H) 12,30 (s, 1H, N-H)MS (ES+) : m/z 452.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-(4-methoxyphenyl)piperazine-1-carbodithioate (5d)

Yield: %84. M.p. 193-195 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3295 (amide N-H), 1658 (amide C=O), 1554-1658 (C=C , C=N) 1218(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3) 3.15(s,3H,OCH_3)2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,19(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7.90(d,2H,Ar-H),8.12 (d,2H,Ar-H) 12.15 (s, 1H, N-H)MS (ES+) : m/z 437.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-benzylpiperazine-1-carbodithioate(5e)

Yield: %70. M.p. 177-179 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3276 (amide N-H), 1655 (amide C=O), 1544-1652 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3)3.42(s,2H,CH2), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4, 20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$), 7.83(d,2H,Ar-H),7.90 (d,2H,Ar-H) 12.18 (s, 1H, N-H)MS (ES+) : m/z 421.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-(4-methylbenzyl)piperazine-1-carbodithioate(5f)

Yield: %74. M.p. 166-168 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3270 (amide N-H), 1652 (amide C=O), 1552-1650(C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ2.28(s,3H,CH3), 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3)3.42(s,2H,CH2), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7.83(d,2H,Ar-H),7.90 (d,2H,Ar-H) 12.24 (s, 1H, N-H)MS (ES+) : m/z 435.

2-((4,5-Dimethylthiazol-2-yl) amino)--2-oxoethyl 4-(pyrimidin-2-yl)piperazine-1-carbodithioate(5g)

Yield: %76. M.p. 154-156 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3286 (amide N-H), 1683 (amide C=O), 1544-1652 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2,24 (s,3H,thiazole5-CH_3), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),6.58(t,1H,Ar-H),7.56 (d,2H,Ar-H) 12.32 (s, 1H, N-H)MS (ES+) : m/z 409.

2-((4-methyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-phenylpiperazine-1-carbodithioate(5h)

Yield: %66. M.p. 144-146 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3270 (amide N-H), 1668(amide C=O), 1466-1551 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,19(twobs, 4H, piperazine C2.6-H),4.32(s,2H,CO$\underline{CH_2}$),7.66 (m,3H,Ar-H),7.47(d,2H,Ar-H),7.25(m,3H,Ar-H), 7.40(d,2H,Ar-H) 12.41 (s, 1H, N-H)MS (ES+) : m/z 469.

2-(4-Dimethyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-(4-fluorophenyl)piperazine-1-carbodithioate(5i)

Yield: %67. M.p. 186-188 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3260 (amide N-H), 1710 (amide C=O), 1463-1550 (C=C , C=N)

1300(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H), 4.32 (s,2H,CO$\underline{CH_2}$),7,74(d,2H,Ar-H),7.83(d,2H,Ar-H), 7.32 (m, 3H, Ar-H), 7.42(d,2H,Ar-H) 12.42 (s, 1H, N-H)MS (ES+) : m/z 487.

2-((4-methyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate(5j)

Yield: %70. M.p. 155-157 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3287(amide N-H), 1650 (amide C=O), 1444-1556 (C=C , C=N) 1230(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20 (twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$), 7.89(d,2H,Ar-H), 8.10 (d,2H,Ar-H), 7.25 (m,3H,Ar-H), 7.40 (d,2H,Ar-H) 12.38 (s, 1H, N-H) MS (ES+) : m/z 414.

2-((4methyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-(4-methoxyphenyl)piperazine-1-carbodithioate(5k)

Yield: %64. M.p. 190-192 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3290 (amide N-H), 1654 (amide C=O), 1554-1658 (C=C , C=N) 1218(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 3.15(s,3H,OCH_3)2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,19(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7.90(d,2H,Ar-H),8.12 (d,2H,Ar-H) , 7,28(m,3H,Ar-H),7.42(d,2H,Ar-H)12.44 (s, 1H, N-H)MS (ES+) : m/z 499.

2-((4methyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-benzylpiperazine-1-carbodithioate(5l)

Yield: %73. M.p. 166-168 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3288 (amide N-H), 1675 (amide C=O), 1544-1654 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 3.42(s,2H,CH2), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7.83(d,2H,Ar-H),7.90 (d,2H,Ar-H) 7,40(m,3H,Ar-H),7.52(d,2H,Ar-H)12.42 (s, 1H, N-H)MS (ES+) : m/z 483.

2-((4methyl-5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-(4-methylbenzyl)piperazine-1-carbodithioate(5m)

Yield: %84. M.p. 166-168 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3270 (amide N-H), 1652 (amide C=O), 1552-1650(C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ2.28(s,3H,CH3), 2.16 (s, 3H,thiazole 4-CH_3), 3.42(s,2H,CH_2), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$),7.83(d,2H,Ar-H),7.90 (d,2H,Ar-H) 7,16(m,3H,Ar-H),7.24(d,2H,Ar-H) 12.41 (s, 1H, N-H)MS (ES+) : m/z 497.

2-((4-methyl -5-phenylthiazol-2-yl) amino)--2-oxoethyl 4-(pyrimidin-2-yl)piperazine-1-carbodithioate(5n)

Yield: %76. M.p. 154-156 ^{o}C. IR (KBr) ν_{max} (cm^{-1}): 3286 (amide N-H), 1683 (amide C=O), 1544-1652 (C=C , C=N) 1204(C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H,thiazole 4-CH_3), 2.51(t, 4H, piperazine C3,5-H), 3.94 and 4,20(twobs, 4H, piperazine C2,6-H),4.32(s,2H,CO$\underline{CH_2}$), 6.58 (t,1H,Ar-H), 7.56 (d,2H,Ar-H) 7, 30(m,3H,Ar-H), 7.40(d,2H,Ar-H) 12.40 (s, 1H, N-H) MS (ES**+**) : m/z 471.

Antimicrobial Activity

The antimicrobial activities of compounds were tested using the microbroth dilution method.[24] Tested microorganism strains were *Micrococcus luteus* (NRLL B-4375), *Bacillus subtilis* (NRS-744), *Pseudomonas aeruginosa* (ATCC-254992), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (ATCC-25922), *Listeria monocytogenes* (ATCC-7644). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Hundred microliter of each microorganism suspension was then added into the wells. The last well-chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18-24 h, antimicrobial activity was detected by spraying of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining. Streptomycin was used as standard antibacterial agent as shown in table 1.

Table 1: Antibacterial activities of the compounds as MIC values (µg/mL)

Compound	A	B	C	D	E	F
5a	250	250	250	250	250	250
5b	250	250	250	250	250	125
5c	250	250	250	125	250	125
5d	250	250	250	250	250	125
5e	250	250	500	250	500	125
5f	250	250	500	250	500	125
5g	250	250	250	250	250	250
5h	250	250	250	250	500	250
5i	250	250	250	250	250	125
5j	250	250	500	125	500	125
5k	250	250	250	250	250	125
5l	250	250	250	250	250	250
5m	250	250	500	250	250	250
5n	250	250	250	250	250	250
Streptomyicine	7.81	15.625	15.625	31.25	31.25	125

A: *L. monocytogenes* (ATCC-7644), B: *M. luteus* (NRLL B-4375), C: *B. subtilis* (NRS-744), D: *E. coli* (ATCC-25922), E: *S. aureus* (NRRL B-767), F: *P. aeruginosa* (ATCC-254992).

Minimum Fungicidal Concentration

The antifungal activities of the synthesized compounds were tested using the microbroth dilution method with

some modifications.[24,25] Tested fungal strains were *Aspergillus parasiticus* (NRRL-465), *Aspergillus flavus* (NRRL-980), *Aspergillus niger* (ATCC-1094), *Fusarium solani* (NRRL-13414), *Candida glabrata* (Clinical Isolate, Eskişehir Osmangazi University, Faculty of Medicine, Eskişehir, Turkey), *C. tropicalis* (NRLL-Y-12968), *C. krusei* (NRLL-Y-7179), *C. parapsilosis* (NRLL-Y-12696). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Filamentous fungal strains grown on PDA at 25°C for 5 suspensions in double-strength Potato Dextrose Broth (PDB) were standardized to 10^5 spores/mL. Overnight-grown on Sabouraud Dextrose Agar (SDA) at 25 ºC for 5 days suspensions in double-strength Mueller-Hinton broth were standardized to 10^8 CFU/mL using McFarland No: 0.5 standard solutions. Hundred microliter of each cell suspension was then added into the wells. The last well-chain without a fungus was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 25°C for 48-72 h, antifungal activity was detected by investigation of mycelia growing under stereo microscope. Minimum fungicidal concentration (MFC) was defined as the lowest concentration of compounds that inhibited visible mycelia growth for filamentous fungi and turbidity for yeasts. Ketoconazole was used as an antifungal agent as shown in table 2.

Table 2: Antifungal activities of the compounds as MIC values (µg/mL)

Compound	A	B	C	D	E	F	G	H
5a	250	250	250	250	250	250	250	250
5b	250	250	250	250	250	250	250	250
5c	250	250	250	250	250	250	250	250
5d	250	250	250	250	250	250	250	250
5e	250	250	250	250	250	250	250	250
5f	250	250	250	250	250	250	250	250
5g	250	250	250	250	250	250	250	250
5h	250	250	250	250	250	250	250	250
5i	250	250	250	250	250	250	250	250
5j	250	250	250	250	250	250	250	250
5k	250	250	250	250	250	250	250	250
5l	250	250	250	250	250	250	250	250
5m	250	250	250	250	250	250	250	250
5n	250	250	250	250	250	250	250	250
Ketoconazole	31.25	15.62	7.81	62.5	31.25	7.81	62.5	7.81

A: *A. niger* (ATCC 1095), **B**: *A. flavus* (NRRL 980), **C**: *A. parasiticus* (NRRL 465), **D**: *F. solani* (NRRL 13414), **E**: *C. glabrata* (Clinical Isolate, Eskişehir Osmangazi University, Faculty of Medicine, Eskişehir, Turkey), **F**: *C. tropicalis* (NRLL-Y-12968), **G:** *C. krusei* (NRLL-Y-7179), **H:** *C. parapsilosis* (NRLL-Y-12696).

AChE Inhibition

All compounds were subjected to a modified method of Ellman's test[26] in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5, 5-dithio-bis (2-nitrobenzoic) acid (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and Donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (**5a-5n**) were measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control.[27]

Enzymatic assay

Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2% DMSO at a concentration range of 10^{-1}-10^{-6} mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB) (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate.

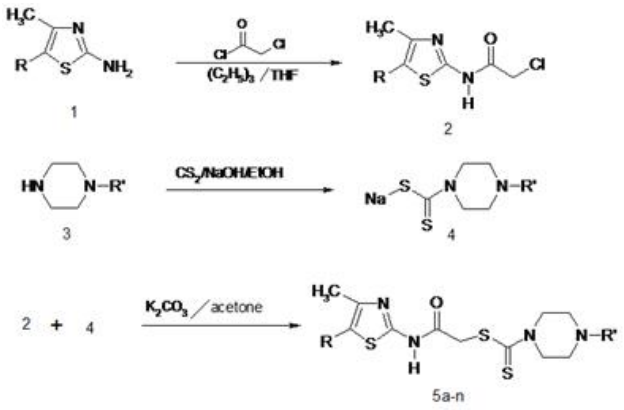
All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

$$\text{Inhibition \%} = (A_C - A_I) / A_C \times 100$$

Where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as Mean ± SD.

RESULTS AND DISCUSSION

Initially, 2-chloroacetylamino-5-substituted-4-methylthiazole (2) was obtained by the reaction of 5-substitued-2-amino -4-methylthiazole with chloroacetyl chloride in the presence of triethylamine. In the present work fourteen new compounds were synthesized, the reaction of 2-chloroacetylamino-5-substituted-4-methylthiazole (2) with appropriate sodium salts of N-substituted piperazine dithiocarbamoic acids (4) resulted in N-substituted piperazine dithiocarbamoic acid, 2-((5-substituted-4-methylthiazol-2-yl) amino)-2-oxoethyl 4-substitutedpiperazine-1-carbodithioate derivatives (5). The reactions are summarized in the following scheme.



R'	R	Compound
Phenyl	CH_3	5a
4-fluorophenyl	CH_3	5b
4-nitrophenyl	CH_3	5c
4-methoxyphenyl	CH_3	5d
Benzyl	CH_3	5e
4-methylbenzyl	CH_3	5f
2-pyrimidinyl	CH_3	5g
Phenyl	phenyl	5h
4-fluorophenyl	phenyl	5i
4-nitrophenyl	phenyl	5j
4-methoxyphenyl	phenyl	5k
Benzyl	phenyl	5l
4-methylbenzyl	phenyl	5m
2-pyrimidinyl	phenyl	5n

The structures of the synthesized compounds (5a-n) were elucidated by spectral data. In the IR spectra of the compounds characteristic stretching bands for C=O,C=S and N-H groups were observed at 1652-1680,1204-1300 cm^{-1} and at 3260-3295 cm^{-1}, respectively. These are common bands which are present in all the synthesized final compounds. In the ^{1}H-NMR spectra of the compounds, the signal due to $COCH_2$ methylene protons present in the all compounds,appeared at 4.32 ppm,as singlest.. protons of the piperazine ring were seen at the range of 2.51 ppm and 4.20 ppm as broad singlets. Aromatic protons appeared at7.34-8.10 ppm region and NH protons were elucidated at 12.15-12.42 region as expected The mass spectra of the compounds showed (M+1) peaks in agreement with their molecular weight.

Table 3: % AChE inbition of the compounds and IC_{50} values

Compound	AChE Inhibition (%)		
	1 mM	0.1 mM	IC_{50} (mM)
5a	18.69±0.68	12.68±2.32	> 1
5b	36.01±2.03	20.53±2.83	> 1
5c	5.01±1.29	2,44±2.22	> 1
5d	16.41±1.98	2.35±2.56	> 1
5e	23.11±3.60	2.96±3.07	> 1
5f	13.06±0.45	12.44±2.34	> 1
5g	23.80±2.54	9.39±3.05	> 1
5h	4.72±2,56	2.91±2,22	> 1
5i	12.56±1,34	ND	ND
5j	27.64±1.96	ND	ND
5k	27.85±1.54	7.33±2.60	> 1
5l	22.43±8.44	12.86±1.91	> 1
5m	14.81±3.70	ND	ND
5n	6.60±1.65	ND	ND
Donepezil	99,01±4,89	95,52±5.01	0.054±0.002 (µM)

The compounds were tested *in vitro* against various pathogenic bacteria and fungi species. Among bacteria species, *P. aeruginosa* was the most susceptible bacterium to compounds **5b,5c,5d,5e,5f,5i,5j and 5k.** These compounds and streptomycin exhibited the same level of antibacterial activity with a MIC value of 125 µg/mL, whereas other derivatives showed their antibacterial activity against *P. aeruginosa* with a MIC value of 250 µg/mL. Among compounds **5a-n** compound **5c and 5j** bearing 4-nitrophenyl was found to be the most potent antibacterial agent against *E. coli* with a MIC value of 125 µg/mL when compared with streptomycin (MIC= 31.25 µg/mL).All compounds exhibited the inhibitory activity against *F. solani* and *C. krusei* with a MIC value of 250 µg/mL, whereas ketoconazole showed its inhibitory activity with a MIC value of 62.5 µg/mL. Final products (**5a-n**) were tested for their *in vitro* growth inhibitory

activity against human pathogens. data of the compounds and the reference drugs are given in Table 1 and table 2.

ACHE Inhibitory Activity

The synthesized compounds (5a-n) were tested for their ACHE inhibitory activities by slightly modified Ellman's assay as described in experimental section, Initially, all compounds were tested for their inhibition potency against ACHE at 0,1-1µM doses, Donepezil was used as a control agent. The IC_{50} values could not be defined of the tested compounds and were reported by comparing with standard drug Donepezil. % ACHE inhibition values of the tested compounds and control reference substance are summarized in table 3.

REFERENCES

1. Ates O, Kocabalkanli A, Cesur N, Otuk G, Synthesis and antimicrobial activity of some 5-aryl-2-[(N,N-disubstituted thiocarbamoylthio)acylamino]-1,3,4oxadiazoles, IL Farmaco., 53, 1998, 541-544.
2. Hurt S, Ollinger J, Arce G, Bui Q, Tobia AJ, van Raven sway B, Dialkyl dithiocarbamates (EBDCs) In Hayes' Handbook of pesticide Toxicology principles: 2nd ed: Robert Krieger, Ed. Acdemic Press: san Diego, USA, 2001, 1759-1781.
3. Duan YC, Zheng YC, Li X.C. Wang, M.M. Ye, X.W. Guan, Y.Y. Liu, G.Z. Zheng, J.X. Liu, H.M. Design, Synthesis and anti proliferative activity studies of novel 1,2,3 triazoleedithiocarbamateeurea hybrids, Eur.J. Med.Chem., 64, 2013, 99-110.
4. Jain AV, Analysis of organophosphate and carbamate pesticides and anticholinesterase therapeutic agents, In: Toxicology of organophosphate and carbamate compounds: R.C. Gupta, Ed: Elsevier: Amsterdam, 2006, 681-701.
5. Altintop MD, Gurkan-Alp, AS Ozkay, Y. Kaplancikli ZA, Synthesis and biological evaluation of a series of dithiocarbamates as new cholinesterase inhibitors, Arch.Pharm.Chem.Life Sci., 346, 2013, 571-576.
6. Turan Zitouni, G.Ozdemir, A.Kaplancikli, Z.A. Synthesis and Antimicrobial Activities of Some1-[(N,N-Disubstitutedthiocarbamoylthio)acetyl]-3,5-diaryl-2-pyrazoline-Phosphours, Sulfur, and Silicon, 180, 2005, 2717-2727.
7. Turan-Zitouni G, Ozdemir A, Guven K, Synthesis of some 1-[(N,N disunstitutedthiocarbamoyl]thio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline activities, Arch, Pharm.Med.Chem., 338, 2005, 96-104.
8. Kaplancikli ZA, Turan-Zitouni G, Revial G, Synthesis of Some Dithiocarbamate Derivatives And Their Antimicrobial Activity, Phosphours, Sulfur, and Silicon, 179, 2004, 1449-1454.
9. Karaburun AC, Kaplancikli ZA, Gundogdu-Karaburun N, Demirci F, Synthesis, Antibacterial and Antifungal Activities of Some Carbazole Dithiocarbamate Derivatives Lett. Drug Des.Discov., 8, 2011, 811-815.
10. Kashyap SJ, Garg VK, Sharma PK, Kumar N, Dudhe R, Gupta JK, Thiazole: having diverse biological activities, Med.Chem.Res., 21, 2012 , 2123-2132.
11. Bharti SK, Nath G, Tilak R, Singh SK, Synthesis, antibacterial and anti-fungal activities of some Schiff bases containing 2,4-disubstituted thiazole ring, Eur.J. Med.Chem., 45, 2010, 651-660.
12. Bondock S, Naser T, Ammarb YA, Synthesis of some new 2-(3-pyridyl)-4,5 disubstituted thiazoles as potent antimicrobial agents, Eur.J. Med.Chem., 62, 2013, 270-279.
13. Desai NC, Bhatt N, Somani H, Trivedi A, Synthesis, antimicrobial and cytotoxic of some thiazole clubbed 1,3,4oxadiazoles, Eur.J. Med.Chem., 67, 2013, 54-59.
14. Padmavathi V, Prema Kumari C, Venkatesh BC, Padmaja A, Synthesis and antimicrobial activity of amido linked pyrrolyl and pyrazolyl-oxazoles, thiazoles, Eur.J. Med.Chem., 46, 2011, 5317-5326.
15. Bondock S, Fadaly W, Metwally MA, Synthesis and antimicrobial activity of some thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety, Eur.J. Med.Chem., 45, 2010, 3692-3701.
16. Karegoudar P, Karthikeyan MS, Prasad DJ, Mahaling M, Holla BS, Kumari NS, Synthesis of some novel 2,4 disubstituted thiazoles as possible antimicrobial agents, Eur.J. Med.Chem., 43, 2008, 261-267.
17. Andreani A, Burnelli S, Granaiola M, Guardigli M, Leomi A, Locatelli A, Morigi R, Rambaldi M, Rizzoli M, Varoli L, Roda A, Chemiluminescent high-throughput micro assay applied to imidazo[2,1-b]thiazole derivatives as potential acetylcholinesterase and butyrylcholinesterase inhibitors, Eur.J. Med.Chem., 43, 2008, 657-661.
18. Sivakumar S, Kumar RR, Ali MA, Choon TS, An atom economic synthesis and AChE inhibitory activity of novel dispiro-7-aryltetrahydro-1Hpyrro;o[1,2-c][1,3]thiazole and -4-aryloctahydroindolizine-Nmethylpiperidin-4-one hybrid heterocycles, Eur.J. Med.Chem., 65, 2013, 240-248.
19. Ali MA, Ismail R, Choon TS, Kumar RS, Osman H, Arumugam N, Almansour AI, Elumalai K, Singh A, AchE inhibitor: A regio-and stereo-selective 1,3dipolar cycloaddition for the synthesis of novel substituted 5,6dimethoxy spiro[5,3']oxindolespiro[6,3'']-2,3dihydro-1Hinden-1''one-7-(substitutedaryl)-tetrahydro-1Hpyrrolo[1,2-c][1,3]thiazole, Bioorg. Med. Chem.Lett. 22, 2012, 508-511.
20. Imramovsky A, Pejchal V, Stepankova S, Vorcakova K, Jampilek J, Vanco J, Simunek P, Kralovec K, Bruckova L, Mandikova J, Trejtnar F, Synthesis and invitro evalution of new derivatives of 2-substituted-6-fluorobenzo[d]thiazole as cholinesterase inhibitors, Bioorg. Med. Chem., 21, 2013, 1735-1748.
21. Rahman AU, Choudhary MI, Bioactive natural products as a potential source of new pharmacophores-a theory of memory, Pure Appl.Chem., 73, 2001, 555-560.
22. Singh N, Sharma US, Sutar N, Kumar S, Sharma UK, Synthesis and antimicrobial activity of some novel 2-aminothiazole derivatives, J. Chem.Pharm.Res., 2(3), 2010, 691-698.
23. Karali N, Apek I, Ozkirimli S, Gursoy A, Dogan SU, Eraslan A, Ozdemir O, Synthesis and pharmacology of new dithiocarbamic asid esters derived from phenothiazine and diphenylamine, Arch.Pharm.Pharm.Med.Chem., 332, 1999, 422-426.

24. W Winn, S Allen, W Janda, E Koneman, G Procop, P Schreckenberger G, Woods in Color Atlas and Textbook of Diagnostic Microbiology, Lippincott Williams & Wilkins, 6th Ed., Baltimore and Philadelphia, 2006.

25. A Espinel-Ingroff, *In Vitro* Fungicidal Activities of Voriconazole, Itraconazole, and Amphotericin B against Opportunistic Moniliaceous and Dematiaceous Fungi, J. Clin. Microbiol., 39(3), 2001, 954-958.

26. Perry NS, Houghton PJ, Theobald A, Jenner P, Perry EK, In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes, J Pharm Pharmacol., 52, 2000, 895–902.

27. Ellman GL, courtney KD, Andres V Jr, Feather-Stone RM, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem Pharmacol., 7, 1961, 88–95.

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