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Synthesis, anticandidal activity, and cytotoxicity of some thiazole derivatives with dithiocarbamate side chains

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Abstract: Some thiazole derivatives bearing dithiocarbamic acid esters were synthesized in order to investigate their anticandidal activity and cytotoxicity. The structures of the obtained final compounds (**6a**–**j**) were confirmed by spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) and elemental analysis. The anticandidal activity of the compounds was determined (**6a**–**j**) using the microbroth dilution method and their cytotoxicity was evaluated according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against normal cells. Contrary to expectations, weak antifungal activity was observed with IC_{50} values ranging between 30 and 403 μg/mL.

Key words: Thiazole, dithiocarbamate, anticandidal activity, cytotoxicity

1. Introduction

Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases particularly in patients undergoing anticancer chemotherapy, organ transplants, or long treatment with antimicrobial agents and in patients with AIDS because of immune system suppression. Such a broad range of infections and development of resistance to currently available antifungal agents require an equally broad range of diagnostic and therapeutic strategies.[1,2]

The fungicidal activities of sulfurated compounds have been known for a long time.[3] Dithiocarbamates, which are an important class of sulfur-containing compounds, were also described as herbicides and fungicides, previously.[4–6] First, Kligman and Rosenweig determined the activity of 4 dimethyl dithiocarbamate salts against several pathogenic fungi and commented on their possible application in human therapy.[7,8] In the last decade, dithiocarbamate moiety combined with different heterocyclic ring systems was studied widely, and now these compounds form a promising group of novel antifungal agents.[9] The dithiocarbamate-including compounds are known to act as inhibitors of enzymes and have a profound effect on biological systems, because of their strong metal-binding capacity. The well-known thiocarbamate class of antifungal drug tolciclate (**I**) and the fungicidal active plant defense agent brassinin (**II**) are famous sulfurated compounds. Additionally,

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rhodanine (**III**) and its derivatives are the other dithiocarbamate-including molecules known for their ability to inhibit fungal protein mannosyl transferase 1 (PMT1), which plays a key role in the biosynthesis of the fungal cell wall of *Candida* (Figure).[10,11]

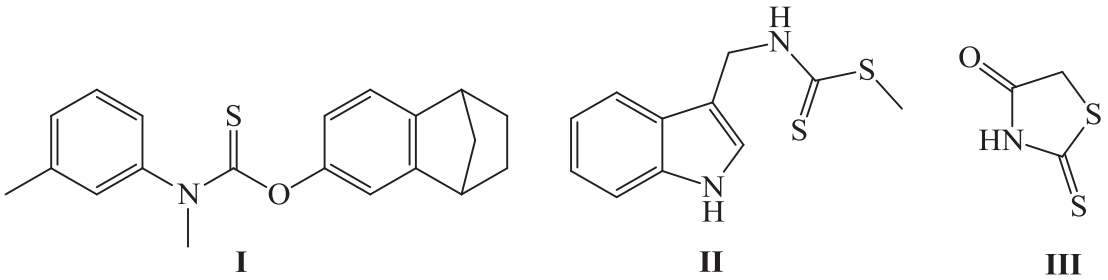


Figure. The chemical structures of the tolciclate (I), brassinin (II), and rhodanine (III).

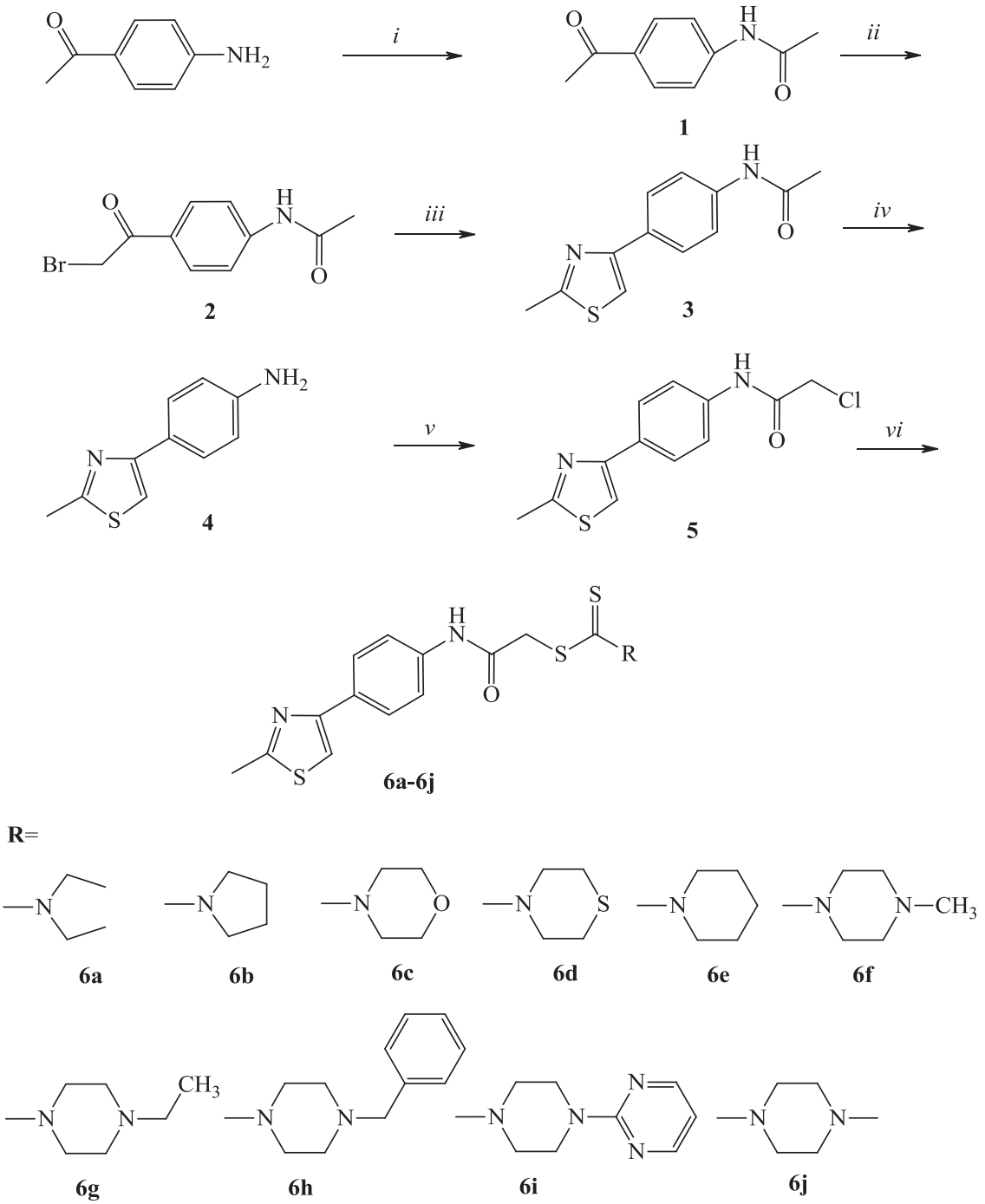
Thiazoles have a prominent position among heterocycles and they can be obtained from microbial and marine origins as well as by synthetic procedure.[12,13] The thiazole ring is present in many biologically active compounds and drugs such as thiamine (vitamin B1), abafungin (antifungal),[14] bleomycin and tiazofurin (antineoplastic agents), ritonavir (anti-HIV drug), fanetizole and meloxicam (anti-inflammatory agents), nizatidine (antiulcer agent), imidacloprid (insecticide),[15] myxothiazols (fungicide),[16] melithiazols (fungicide),[17] and penicillin (antibiotic). Besides these bioactive compounds, there are a lot of studies about thiazole derivatives with antifungal properties in the literature.[18–20]

According to the foregoing literature survey, we now report the synthesis of the dithiocarbamic acid derivatives of *N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide structure with potential anticandidal activity and cytotoxicity in this study.

2. Results and discussion

The present study was undertaken to synthesize some thiazole derivatives bearing dithiocarbamic acid ester and to investigate their anticandidal activity and cytotoxicity. The target compounds were obtained in multistep organic synthesis as shown in the Scheme. The initial compound 4-aminoacetophenone in a TEA/THF mixture was acetylated with chloroacetyl chloride to obtain 4-(acetylamino)acetophenone (**1**); then compound **1** in AcOH was brominated to obtain *N*-[4-(2-bromoacetyl)phenyl]acetamide (**2**). The obtained amide compound (**2**) was reacted with thioacetamide to give *N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (**3**). After hydrolysis of the acetyl group on amino moiety, compound 4-(2-methyl-4-thiazolyl)aniline (**4**) was synthesized, which was then acetylated with chloroacetyl chloride to obtain 2-chloro-*N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (**5**). In the final step, compound **5** was reacted with appropriate dithiocarbamate salts to give the final compounds (**6a**–**6j**). Compound **6c** (2-[[4-(2-methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl morpholine-4-carbodithioate) was synthesized and registered with the chemical abstract service before, but there are no scientific data about the molecule and so we included this compound in our research.

The structures of the synthesized compounds were elucidated by spectral data and elemental analysis, and significant stretching bands in the IR spectra were observed in the expected regions. Stretching bands for C=O and N–H groups were observed at 1665–1683 cm^{-1} and 3266–3290 cm^{-1}, respectively. In the ^1H NMR spectra of the compounds, methyl protons at the second position of the thiazole ring and N–H protons belonging to amide moiety were observed at about 2.67–2.76 ppm and 9.05–10.37 ppm. Unexpectedly, C_5–H of



Scheme. The synthesis of the compounds (**6a**–**j**). Reagents: (i) acetyl chloride, TEA, THF, 0–5 °C; (ii) Br_2, AcOH; (iii) thioacetamide, EtOH, r.t. (iv) 10% HCl, EtOH, reflux; (v) chloroacetyl chloride, TEA, THF, r.t.; (vi) appropriate sodium salts of N,N-disubstituted dithiocarbamic acids, K_2CO_3, acetone, reflux.

the thiazole ring was observed at about 7.23–7.27 ppm and as 2 singlets that are thought to be due to magnetic anisotropy. Additionally, protons of the $-CH_2$ group linked to the sulfur atom were observed at 4.22–4.26 ppm as singlets and protons of the cyclic structures were seen at 1.73 ppm and 4.65 ppm as broad singlets,

commonly. The other peaks belonging to aromatic and aliphatic protons were observed in the estimated areas. The ^{13}C NMR spectra gave the expected data for entire carbons in the target compounds. $COCH_2$ group peaks were observed at 4.42–4.46 ppm for CH_2 and at 166–168 ppm for C=O carbons. C=S carbons gave peaks at 191–197 ppm. C=N carbons of the thiazole ring were recorded at 156–157 ppm. The mass spectra (EI-MS) of the compounds showed (M+1) peaks in agreement with their molecular weight. Elemental analysis results for C, H, and N elements were satisfactory within calculated values of the compounds.

The target compounds **6a**–**j** were screened for their in vitro anticandidal activity against 7 candida species, including standard strains and clinical isolates. MIC is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the reference drug ketoconazole are given. The results provided in Table 1 indicate that most of the prepared compounds displayed broad antifungal spectra with MIC values ranging from 62.5 to 125 μg/mL against all the tested strains. Among all evaluated strains, the compounds **6a**, **6b**, **6c**, **6f**, and **6g** had anticandidal activity lower than that of the standard drug. Compound **6a** inhibited all species at a concentration of 62.5 μg/mL except against *C. tropicalis.* Both of the compounds **6b** and **6c** displayed anticandidal activity against *C. albicans* (ATCC 90028) and *C. glabrata* (isolate 1) at the same concentrations (62.5 μg/mL). Compounds were also studied for their cytotoxic properties using the MTT assay. The IC_{50} (μg/mL) values of the compounds against NIH/3T3 cells are shown in Table 2. The biological study indicated that compound **6f** possessed the highest cytotoxicity, with a value of about 30 μg/mL, whereas compound **6i** exhibited the lowest cytotoxicity, with a value of about 330 μg/mL, against NIH/3T3 cells.

Table 1. Anticandidal activity of the compounds (MIC in μg/mL).

Comp.	A	B	C	D	E	F	G
6a	62.5	62.5	62.5	125	62.5	62.5	62.5
6b	125	62.5	62.5	125	125	62.5	125
6c	62.5	62.5	125	125	125	62.5	125
6d	ND	ND	ND	ND	ND	ND	ND
6e	ND	ND	ND	ND	ND	ND	ND
6f	125	125	125	62.5	62.5	125	62.5
6g	62.5	125	125	125	125	62.5	62.5
6h	ND	ND	ND	ND	ND	ND	ND
6i	ND	ND	ND	ND	ND	ND	ND
6j	ND	ND	ND	ND	ND	ND	ND
Ref.	0.78	1.56	0.78	1.56	0.39	0.39	0.39

Ref.: Ketoconazole, ND: Not defined

A: *C. albicans* (isolate, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey), B: *C. glabrata* (isolate 1, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey), C: *C. utilis* (NRRLY-900), D: *C. tropicalis* (NRRLY-12968), E: *C. krusei* (NRRLY-7179), F: *C. albicans* (ATCC 90028), G: *C. glabrata* (isolate 2, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey).

3. Conclusion

In this study, we report the synthesis, spectral studies, and biological evaluation of some thiazole derivatives bearing dithiocarbamic acid ester (**6a**–**j**). The structures proposed for the synthesized compounds (**6a**–**j**) are

well supported by spectroscopic data and elemental analysis. Some of the final compounds (**6a**, **6b**, **6c**, **6f**, and **6g**) were evaluated for their anticandidal activity and they exhibited weak activity against all tested strains. The cytotoxicity of the compounds was also studied and compounds **6c**, **6d**, **6h**, and **6i** displayed the lowest cytotoxicity against NIH/3T3 cells.

Table 2. In vitro cytotoxicity of the compounds.

Compound	$IC_{50}(\mu g/mL)^a$
6a	73 ± 12
6b	92 ± 18
6c	117 ± 71
6d	262 ± 9
6e	133 ± 11
6f	30 ± 5
6g	81 ± 3
6h	403 ± 8
6i	330 ± 44
6j	133 ± 25

[a] Cytotoxicity of the compounds to mouse fibroblast (NIH/3T3) cell line. Incubation for 24 h. IC_{50} is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± standard deviation of triplicate determinations.

4. Experimental

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All melting points (mps) 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); NMR, VARIAN Mercury 400 FT spectrometer (Varian Inc, Palo Alto, CA, USA) in $CDCl_3$ using TMS as internal standard; M+1 peaks were determined by AB Sciex-3200 Q-TRAP LC/MS/MS system (Applied Biosystems Co., MA, USA).

4.1. 4'-Acetaminoacetophenone (1)

4'-Aminoacetophenone (0.05 mol, 6.75 g) and triethylamine (0.06 mol, 8.34 mL) were dissolved in THF (100 mL) with a constant stirring at 0–5 °C; then acetyl chloride (0.06 mol, 4.78 mL) was added dropwise to this solution. The reaction mixture was stirred for 1 h at room temperature. After evaporation of solvent, the obtained solid was washed with water, filtered, dried, and recrystallized from ethanol. Yield: 82%; mp 168 °C (reference 168–170 °C).[21]

4.2. 4-(2-Bromoacetyl)acetanilide (2)

Compound **1** (0.04 mol, 7.08 g) and HBr (0.5 mL) were dissolved in acetic acid (30 mL) and bromine (0.044 mol, 2.27 mL) was added dropwise at room temperature. After completion of the addition of bromine, the reaction mixture was stirred for 1 h and then poured into ice-water (100 mL). The precipitated product was filtered, washed with water, dried, and then recrystallized from ethanol. Yield: 86%; mp 188 °C (reference

185–187 °C).[22] IR: (KBr) ν_{max} (cm^{-1}): 3363 (amide N–H), 3062 (aromatic C–H), 1698 (ketone C=O), 1665 (amide C=O), 1378–1196 (C–N), and 848 (1,4-disubstituted benzene).

4.3. 4-(2-Methyl-4-thiazolyl)acetanilide (3)

Compound **2** (0.03 mol, 7.68 g) and thioacetamide (0.03 mol, 2.25 g) in ethanol (100 mL) were stirred at room temperature for 48 h. The precipitated product was filtered, dried, and recrystallized from ethanol. Yield: 78%; mp 144 °C (reference 141–142 °C).[23] IR: (KBr) ν_{max} (cm^{-1}): 3365 (amide N–H), 3062 (aromatic C–H), 1665 (amide C=O), 1367–1211 (C–N), and 843 (1,4-disubstituted benzene).

4.4. 4-(2-Methyl-4-thiazolyl)aniline (4)

Compound **3** (0.025 mol, 5.8 g) was refluxed in 10% HCl (100 mL) for 1 h. The mixture was cooled down, poured into ice-water (100 mL) and made basic with 10% NaOH solution. The precipitated product was filtered, dried, and recrystallized from ethanol. Yield: 92%; mp 136 °C (reference mp 133– 135 °C).[23] IR: (KBr) ν_{max} (cm^{-1}): 3365 (amine N–H), 3361 (amine N–H), 3062 (aromatic C–H), 1367–1211 (C–N), and 843 (1,4-disubstituted benzene).

4.5. 2-Chloro-*N*-[4-(2-methyl-4-thiazolyl)phenyl]acetamide (5)

Chloroacetyl chloride (0.02 mol, 1.6 mL) was added dropwise over 15 min to a magnetically stirred solution of compound **4** (0.02 mol, 3.8 g) and triethylamine (0.02 mol, 2.8 mL) in dry THF (15 mL). After completion of the reaction, the solvent was evaporated under reduced pressure. Water was added to wash the resulting solid and the mixture was filtered, dried, and recrystallized from ethanol to give compound **5**. Yield: 83%; mp 156 °C.[24] IR: (KBr) ν_{max} (cm^{-1}): 3367 (amide N–H), 3053 (aromatic C–H), 1676 (amide C=O), 1605–1403 (C=C, C=N), 1369–1214 (C–N), and 838 (1,4-disubstituted benzene). ^{1}H NMR (500 MHz, $CDCl_3$)δ (ppm): 2.69 (3H, s, CH_3), 4.32 (2H, s, CO–CH_2), 7.76 (2H, d, J = 8.2 Hz, Ar–H), 7.81 (1H, s, thiazole C_5–H), 7.87 (d, 2H, J = 8.1 Hz, Ar–H), and 10.50 (s, 1H, N–H).

4.6. General methods for synthesis of compounds 6a–j

Compound **5** (0.001 mol) was stirred with appropriate sodium salts of dithiocarbamic acids (0.0011 mol) in acetone for 3 h. The precipitated product was filtered and washed with water.

4.6.1. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl diethylcarbamodithiodate (6a)

85% yield; mp 95 °C. IR (KBr) ν_{max} (cm^{-1}): 3282 (amide N–H), 1684 (amide C=O), 1310–1005 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30–1.35 (m, 6H, CH_2–$\underline{CH}_3$), 2.76 (s, 3H, C–CH_3), 3.78 (q, J = 7.2 Hz, 2H, $\underline{CH}_2$–CH_3), 4.07 (q, J = 7.6 Hz, 2H, $\underline{CH}_2$–CH_3) 4.23 (s, 2H, CH_2–S), 7.23 and 7.26 (2s, 1H, thiazole C_5–H), 7.57 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.33 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.76, 12.71, 19.54, 40.65, 47.75, 51.12, 111.71, 120.01, 127.08, 130.73, 138.13, 154.91, 166.02, 167.45, 194.90. MS (ES+): m/z 380.

4.6.2. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl pyrrolidine-1-carbodithiodate (6b)

82% yield; mp 158 °C. IR (KBr) ν_{max} (cm^{-1}): 3275 (amide N–H), 1685 (amide C=O), 1332–1019 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00–2.14 (m, 4H, pyrolidine CH_2), 2.75 (s, 3H, C–CH_3), 3.69 (t, J = 6.8 Hz, 2H, pyrrolidine CH_2), 3.97 (t, J = 7.2 Hz, 2H, pyrrolidine CH_2), 4.22 (s, 2H, CH_2–S), 7.23 and 7.26 (2s, 1H, thiazole C_5–H), 7.58 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.36 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.56, 24.55, 26.37, 40.11, 51.39, 56.28, 111.73, 120.02, 127.05, 130.74, 138.12, 154.92, 166.00, 167.39, 191.87. MS (ES+): m/z 378.

4.6.3. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl morpholine-4-carbodithioate (6c)

87% yield; mp 181 °C. IR (KBr) ν_{max} (cm^{-1}): 3279 (amide N–H), 1680 (amide C=O), 1328–1053 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 3H, C–CH_3), 3.79 (brs, 4H, morpholine N–CH_2), 3.97 (brs, 2H, morpholine O–CH_2), 4.26 (s, 2H, CH_2–S), 4.37 (s, 2H, morpholine O–CH_2), 7.24 and 7.27 (2s, 1H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.07 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.33, 0.24, 50.92, 52.56, 65.89, 66.39, 111.62, 119.87, 126.87, 130.71, 137.67, 154.61, 165.83, 166.62, 196.471. MS (ES+): m/z 394.

4.6.4. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethylthiomorpholine-4-carbodithioate (6d)

88% yield; mp 184 °C. IR (KBr) ν_{max} (cm^{-1}): 3286 (amide N–H), 1683 (amide C=O), 1357–1037 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 3H, C–CH_3), 2.78 (brs, 4H, thiomorpholine S–CH_2), 4.26 (s, 2H, CH_2–S), 4.65 (brs, 4H, thiomorpholine N–CH_2), 7.23 and 7.26 (2s, 1H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.05 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.56, 27.60, 40.73, 54.01, 56.03, 111.85, 120.08, 127.10, 130.94, 137.88, 154.83, 166.05, 166.83, 196.02. MS (ES+): m/z 410.

4.6.5. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl piperidine-1-carbodithioate (6e)

86% yield; mp 140 °C. IR (KBr) ν_{max} (cm^{-1}): 3266 (amide N–H), 1665 (amide C=O), 1357–1058 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 (brs, 6H, piperidine CH_2), 2.76 (s, 3H, C–CH_3), 3.91 (brs, 2H, piperidine CH_2), 4.26 (s, 2H, CH_2–S), 4.32 (brs, 2H, piperidine CH_2), 7.23 and 7.27 (2s, 1H, thiazole C_5–H), 7.57 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.28 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.33, 24.06, 25.54, 26.04, 40.46, 52.05, 54.36, 111.51, 119.82, 126.83, 130.53, 137.87, 154.69, 165.78, 167.16, 194.41. MS (ES+): m/z 390.

4.6.6. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-methylpiperazine-1-carbodithioate (6f)

83% yield; mp 167 °C. IR (KBr) ν_{max} (cm^{-1}): 3269 (amide N–H), 1667 (amide C=O), 1346–1023 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H, N–CH_3), 2.53 (brs, 4H, piperazine CH_2), 2.76 (s, 3H, C–CH_3), 3.98 (brs, 2H, piperazine CH_2), 4.23 (s, 2H, CH_2–S), 4.39 (brs, 2H, piperazine CH_2), 7.26 and 7.27 (2s, 1H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.16 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.53, 40.62, 45.74, 50.60, 52.69, 54.50, 111.81, 120.10, 127.08, 130.85, 137.96, 154.85, 166.07, 167.11, 196.02. MS (ES+): m/z 407.

4.6.7. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-ethyllpiperazine-1-carbodithioate (6g)

84% yield; mp 162 °C. IR (KBr) ν_{max} (cm^{-1}): 3285 (amide N–H), 1682 (amide C=O), 1343–1005 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (t, J = 7.2 Hz, 3H, CH_2–$\underline{CH}_3$), 2.43–2.56 (m, 6H, N–CH_2, piperazine CH_2), 2.76 (s, 3H, C–CH_3), 3.98 (s, 2H, piperazine CH_2), 4.26 (s, 2H, CH_2–S), 4.39 (brs, 2H, piperazine CH_2), 7.23 and 7.27 (2s, 1H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.18 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.17, 19.53, 40.61, 50.71, 52.01, 52.27, 52.76, 111.84, 120.11, 127.08, 130.84, 137.98, 154.86, 166.09, 167.15, 195.79. MS (ES+): m/z 421.

4.6.8. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-benzylpiperazine-1-carbodithioate (6h)

89% yield; mp 147 °C. IR (KBr) ν_{max} (cm^{-1}): 3288 (amide N–H), 1666 (amide C=O), 1357–1039 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54–2.59 (m, 4H, piperazine CH_2), 2.76 (s, 3H, C–CH_3), 3.55 (s, 2H, CH_2–C), 3.96 (brs, 2H, piperazine CH_2), 4.25 (s, 2H, S–CH_2), 4.38 (brs, 2H, piperazine CH_2), 7.23–7.33 (m, 6H, Ar–H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 9.16 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.56, 40.60, 50.81, 52.55, 52.91, 62.61, 111.78, 120.07, 127.09, 127.73, 128.68, 129.33, 130.86, 137.17, 137.77, 154.67, 165.79, 166.89, 195.61. MS (ES+): m/z 483.

4.6.9. 2-[[4-(2-Methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl 4-(pyrimidin-2-yl)piperazine-1-carbodithioate (6i)

86% yield; mp 169 °C. IR (KBr) ν_{max} (cm^{-1}): 3285 (amide N–H), 1683 (amide C=O), 1332–1045 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 3H, C–CH_3), 3.99–4.01 (m, 6H, piperazine CH_2), 4.06 (brs, 2H, piperazine CH_2), 4.28 (s, 2H, S–CH_2), 4.64 (brs, 2H, piperazine CH_2), 6.58 (t, J = 4.4 Hz, 1H, Ar–H), 7.23 and 7.27 (2s, 1H, thiazole C_5–H), 7.56 (d, J = 8.4 Hz, 2H, Ar–H), 7.81 (d, J = 8.4 Hz, 2H, Ar–H), 8.35 (d, J = 4.8 Hz, 2H, Ar–H), 9.13 (s, 1H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.32, 40.34, 42.94, 50.10, 52.16, 110.91, 111.58, 119.86, 126.86, 130.66, 137.70, 154.62, 157.84, 161.16, 165.81, 166.71, 196.27. MS (ES+): m/z 471.

4.6.10. Bis{2-[[4-(2-methyl-4-thiazolyl)phenyl]amino]-2-oxoethyl} piperazine-1,4-bis(carbodithioate) (6j)

81% yield; mp 216 °C (decomp.) IR (KBr) ν_{max} (cm^{-1}): 3290 (amide N–H), 1681 (amide C=O), 1352–1011 (C–N and C–O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.67 (s, 6H, C–CH_3), 4.17–4.32 (m, 12 H, S–CH_2, piperazine CH_2), 7.62 (d, J = 8.8 Hz, 2H, Ar–H), 7.76 (s, 2H, thiazole C_5–H), 7.84 (d, J = 8.4 Hz, 4H, Ar–H), 10.37 (s, 2H, N–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.56, 41.96, 44.28, 48.31, 50.29, 113.18, 119.87, 127.12, 130.14, 139.32, 154.24, 165.89, 166.05, 195.60. MS (ES+): m/z 699.

4.7. Anticandidal activity assay

Anticandidal activity of the final compounds was evaluated by the broth microdilution method according to the modified NCCLS M27-A2 standard procedure as indicated in the literature.[25] Tested candida strains and origins

were as follows: *C. albicans* (isolate, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey), *C. glabrata* (isolate 1, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey), *C. utilis* (NRRLY-900), *C. tropicalis* (NRRLY-12968), *C. krusei* (NRRLY-7179), *C. albicans* (ATCC 90028), and *C. glabrata* (isolate 2, obtained from Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey). Ketoconazole was used as positive control and the results (MIC values) are shown in Table 1.

4.8. Cytotoxicity assay

Cytotoxic properties of the compounds were determined by the method mentioned in the literature using mouse embryonic fibroblast (NIH/3T3) cells.[25] The calculated IC_{50} values of the compounds are exhibited in Table 2. The procedure was realized using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[26] NIH/3T3 cells were cultured in 96-well flat-bottom plates at 37 °C for 24 h (2×10^4 cells per well). All the compounds were dissolved in DMSO individually and added to culture wells at varying concentrations (0.5–500 μg/mL); the highest final DMSO concentration was under 0.1%. After 24 h of drug incubation at 37 °C, 20 mL of MTT solution (5 mg/mL MTT powder in PBS) was added to each well. Then a 3-h incubation period was maintained in the same conditions. Purple formazan occurred at the end of the process, which is the reduction product of MTT agent by the mitochondrial dehydrogenase enzyme of intact cells. Formazan crystals were dissolved in 100 mL of DMSO and the absorbance was read by ELISA reader (OD 570 nm). The percentage of viable cells was calculated based on the medium control.

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