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Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dione derivatives

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Abstract—In this study, a series of thiazolyl thiazolidine-2,4-dione derivatives (**Va–f** and **VIa–f**) were synthesized and evaluated for their antibacterial and antifungal activities against *Staphylococcus aureus* (ATCC 25923), methicillin resistant *S. aureus* (MRSA ATCC 43300), methicillin resistant *S. aureus* (MRSA *isolate*), and *Escherichia coli* (ATCC 23556) and *C. albicans* (ATCC10145). All the compounds were found active against used microorganisms.

1. Introduction

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics in human civilization. Bacterial diseases such as tuberculosis, typhus, plague, diphtheria, typhoid fever, cholera, dysentery, and pneumonia have taken a high toll on humanity.[1]

The introduction of antibiotics in the 1940 s was thought to have eliminated the scourge of all infectious diseases. However, due to the widespread use and misuse of antibiotics, bacterial resistance to antibiotics has become a serious public health problem. Some of these resistant strains, such as vancomycin-resistant enterococci (VRE) and multidrug resistant *Staphylococcus aureus* (MRSA), are capable of surviving the effects of most, if not all, antibiotics currently in use.[2–6] With the increase in resistance of bacteria to antibiotic treatment, attention has focused on developing novel approaches to antimicrobial therapy.[7–13]

1,3-Thiazolidines are the new class of antimicrobial agents with activity against broad spectrum of Gram-positive pathogens including *Staphylococci*, *Streptococci*, and *Enterococci*.[14] It has been known that the entrance of arylidene moieties at different positions of the thiazolidine ring enhanced the antimicrobial activity.[15,16] Organic compounds bearing thiazoles of different pharmacodynamic nuclei were found to possess potent antibacterial[17] and antifungal[18] activities.

Previously, we reported that the synthesis and antimicrobial evaluation of 3-substituted-benzyl-5-(4-chloro-2-piperidin-1yl-thiazole-5-yl-methylene)-thiazolidine-2,4-dione derivatives showed a comparable activity to that of ampicillin against *Escherichia coli*.[19] As part of an ongoing research, herein, we have synthesized a new series of thiazolo-substituted phenacyl-2,4-thiazolidinediones **VIa–f** besides the antimicrobial activity and the SAR of a series of newly (**VIa–f**) and previously synthesized thiazolo-substituted benzyl-2,4-thiazolidinedione derivatives **Va–f**[20] were described in order to reveal the lead optimization against the tested various bacteria and the fungus *C. albicans*.

2. Results and discussion

2.1. Chemistry

Thiazolyl-2,4-thiazolidinedione compounds **Va–f**, **VIa–f** were synthesized according to the synthetic pathway described in Scheme 1. 2,4-dichlorothiazole-5-carbalde-

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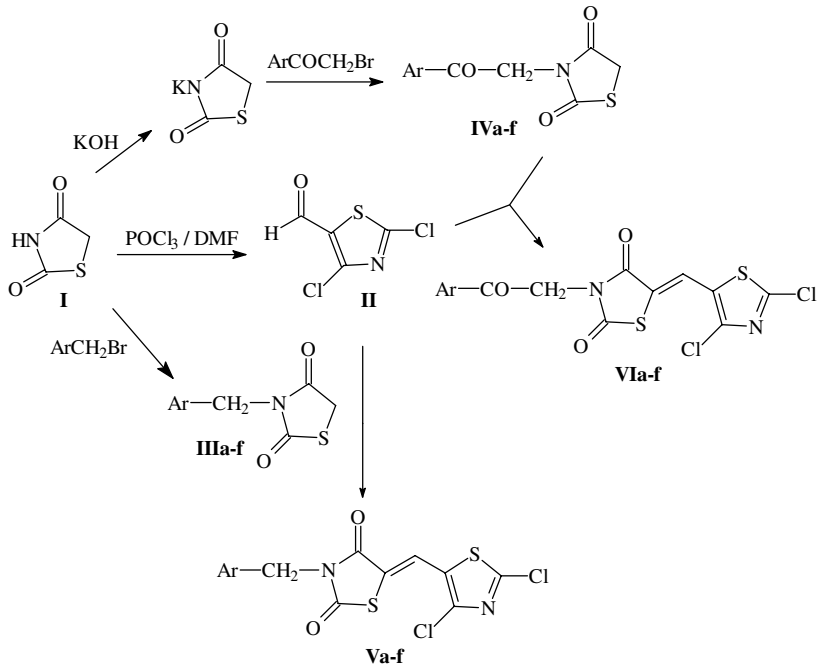


Scheme 1. General synthesis of **Va–f**, **VIa–f**.

hyde (**II**) was obtained with 2,4-TZD[15] (**I**) and *N*,*N*-dimethylformamide in phosphoryl chloride.[21] Substituted benzyl-2,4-thiazolidinediones **IIIa–f** were obtained by 2,4-TZD (**1**) and appropriate benzyl halide derivatives in NaOH/ethanol. Substituted phenacyl-2,4-thiazolidinediones **IVa–f** were synthesized by reacting potassium 2,4-thiazolidinedione with appropriate phenacylbromide derivatives in hot methanol. The condensation of 2,4-dichlorothiazole-5-carbaldehyde **II** with substituted benzyl-2,4-thiazolidinediones **IIIa–f** and phenacyl-2,4-thiazolidinediones **IVa–f** in the presence of sodium acetate/acetic acid glacial by Knoevenagel reaction led to thiazolo-substituted benzyl-2,4-thiazolidinediones **Va–f**[20]and thiazolo-substituted phenacyl-2,4-thiazolidinediones **VIa–f**, respectively.

In the literature, it was reported that in reactions using unsubstituted imidazolidinediones and benzaldehydes in acidic medium, the main product was the *Z* isomer.[22] The coupled ^{13}C NMR study of arylidene thiazolidinediones and imidazolidinediones also showed that the *Z* isomer was formed.[23,24] In this study, only one isomer was obtained except **VIc** and **VIe** having the chloro and dichloro substituents at the *para*-position and *ortho*/*para*-position of the aryl groups at N-3 position of the TZD ring, respectively. The stereoisomers (**VIc** and **VIe**) were isolated using silica gel column chromatography. ^1H NMR spectrums of **VIc-1** and **VIe-1** were very similar to those of **VIc-2** and **VIe-2** except for the signal due to the methine proton, respectively. The methine proton of the **VIc-1** and **VIe-1** appeared at a lower field than that of the **VIc-2** and **VIe-2**. It was reported that the methine proton, deshielded by the adjacent C=O, was detected at 7.70–7.75 ppm in ^1H NMR spectra as observed for analogues 5-arylidene-2,4-TZDs.[25] In *E* isomers, due to the lesser deshielding effect of 1-S of the TZD ring, such a proton should resonate at lower chemical shift values.[26] The calculated values of the methyne protons of **VIc-1**/**VIe-1** and **VIc-2**/**VIe-2** were seen as a singlet at 8.05–8.04 ppm and 7.34–7.33 ppm, respectively.

Furthermore, the X-ray diffractometric analysis of a representative compound **VIc-1** unambiguously confirmed the *Z* configuration at the chiral axis (Fig. 1).

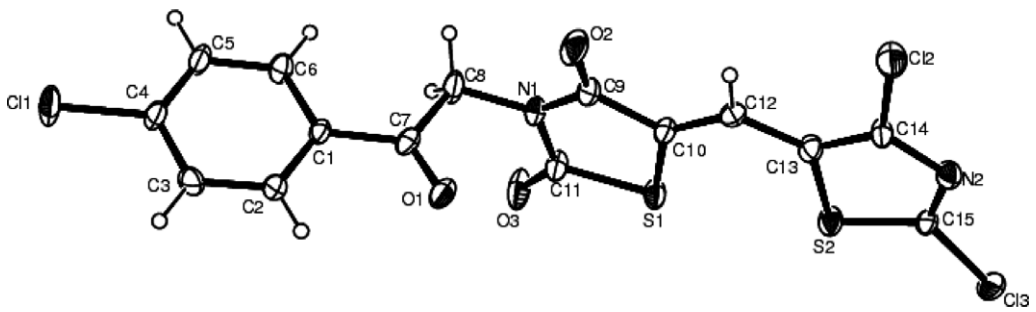


Figure 1. X-ray structure of **VIc-1**.

Methyne protons for compounds **VIa**,**b**,**d**,**f** were seen at 8.04–8.06 ppm as a singlet. In Mass spectra, all the compounds have M+22 ion peak with MS ESI method.

2.2. In vitro antibacterial and antifungal activity

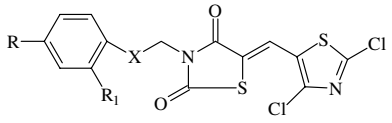
All the newly synthesized thiazolyl-2,4-thiazolidinedione compounds **Va–f**, **VIa–f** were tested for their in vitro antibacterial activity against *S. aureus* (ATCC 25923), methicillin resistant *S. aureus* (MRSA ATCC 43300), methicillin resistant *S. aureus* (MRSA *isolate)* as Gram-positive bacteria, *E. coli* (ATCC 23556) as Gram-negative bacterium, and the antifungal activity was evaluated against *C. albicans* (ATCC10145). The MIC values were determined by twofold serial dilution technique[27] in Mueller–Hinton broth and Sabouraud dextrose agar for the antibacterial and antifungal assays, respectively. In comparison with the antimicrobial activity, ampicillin and ciprofloxacin were used as the reference antibacterial agents, miconazole was used as the reference antifungal agent. All the biological results of the tested compounds are given in Table 1. The combined data were reported that the synthesized compounds **Va–f**, **VIa–f** showing MIC values between 50 and 6.25 μg/ml were able to have an in vitro inhibitory effect against the screened microorganisms.

As seen in Table 1, although none of the compounds showed more activity against methicillin resistant *S. aureus* (MRSA ATCC 43300) than ampicilin, the derivatives **Va–c**, **Vf**, **VIa–VIc-1**, **VId** were found to be significantly potent with a MIC value of 6.25 μg/ml. It can be concluded that a chlorine atom at position R_1 slightly decreased an inhibitory effect. Furthermore, compounds **Vb**, **Vc**, **VIa**, **VIb**, **VIc-1** and **Ve**, **VIe-1**, **VIe-2**, **VIf** showed similar activity against either MRSA (isolate) and MRSA ATCC 43300 with a MIC value of 6.25 and 12.5 μg/ml, respectively. While compounds **Va**, **Vf**, **VId** were more potent against *MRSA ATCC* 43,300, compounds **Vd**, **VIc-2** were more potent against *MRSA* (isolate).

All of the compounds indicated significant inhibitory effect with MIC values between 6.25 and 12.5 μg/ml against *S. aureus* ATCC 23556 as well; suprisingly, the derivatives **Vb–d**, **Vf**, **VIa–VIc-1** showed comparable activity with ampicillin. Structure–activity relationships revealed that attaching an electron-withdrawing group such as halogen or nitro on position R of phenyl ring which has to bind with a methylene bridge instead of acetyl to TZD played a very important role to increase the activity. It should be pointed out that while the configuration of **VIe** did not change the activity against either MRSA ATCC 43300 or *S. aureus* strains, the *Z* configuration of **VIc** was found to have onefold better dilution than *E* configuration. Additionally, the compounds **VIb** and **VId**, which have a fluorine and bromine at the *para*-position of phenacyl groups, respectively, were found to be more active than ampicillin against Gram-negative bacterium *E. coli* ATCC 23556, as for other compounds except **VIc-2** showed similar activity.

All of the derivatives indicated very significant antifungal activity against *C. albicans* ATCC10145, the MICs ranged from 6.25 to 12.5 μg/ml even they were higher than that of standard drug, miconazole. The structure–activity relationships showed that the antifungal activity of the substituents at the phenyl ring is H, Cl, Br,*o*,*p*-di-Cl > F, NO_2 for benzylic 2,4-TZD compounds. As for phenacyl 2,4-TZD compounds, it is Cl,Br > H, F,*o*,*p*-di-Cl, NO_2. This kind of structure could be guide for further antifungal studies.

Table 1. Antibacterial and antifungal activity of the compounds **Va–f** , **VIa–f** (MIC, minimum inhibitory concentration μg/ml)



Compound	X	R	R_1	MRSA ATCC 43300	MRSA (isolate)	*S. aureus* ATCC 25923	*E. coli* ATCC 23556	*C. albicans* ATCC10145
Va	—	H	H	6.25	12.5	12.5	25	6.25
Vb	—	F	H	6.25	6.25	6.25	25	12.5
Vc	—	Cl	H	6.25	6.25	6.25	25	6.25
Vd	—	Br	H	12.5	6.25	6.25	25	6.25
Ve	—	Cl	Cl	12.5	12.5	12.5	25	6.25
Vf	—	NO_2	H	6.25	12.5	6.25	25	12.5
VIa	CO	H	H	6.25	6.25	6.25	25	12.5
VIb	CO	F	H	6.25	6.25	6.25	6.25	12.5
VIc-1	CO	Cl	H	6.25	6.25	6.25	25	6.25
VIc-2	CO	Cl	H	12.5	6.25	12.5	50	6.25
VId	CO	Br	H	6.25	12.5	12.5	6.25	6.25
VIe-1	CO	Cl	Cl	12.5	12.5	12.5	25	12.5
VIe-2	CO	Cl	Cl	12.5	12.5	12.5	25	12.5
VIf	CO	NO_2	H	12.5	12.5	12.5	25	6.25
Miconazole				[a]	[a]	[a]	[a]	25
Ampicillin				1.56	1.56	6.25	25	[a]
Ciprofloxacin				[a]	[a]	[a]	0.78	[a]

[a] No tested.

On the other hand, when we generally glanced at all of the values for *C. albicans* it should be pointed out that isomeric compounds **VIc-1/VIc-2** and **VIe-1/VIe-2** did not show any difference in activity.

3. Conclusion

We report the synthesis and antimicrobial activity of a new series of thiazolyl thiazolidine-2,4-diones (**Va–f** and **VIa–f**). In this study only one isomer was obtained except **VIc** and **VIe** having the chloro and dichloro substituents at the *para*-position and *ortho*/*para*-position of the phenacyl group in phenacyl thiazolyl TZD compounds, respectively. All the compounds were found to be moderately potent against screened microorganisms. In particular, the antifungal effects of all tested compounds were higher than that of standard drug, miconazole. According to this study, it should be pointed out that benzyl or phenacyl groups on the TZD ring did not play a noticeable role for increasing the activity.

4. Experimental

4.1. Chemistry

Melting points were determined with a Büchi SMP-20 melting point apparatus (Essen, Germany) and are uncorrected. All instrumental analyses were performed in Central Lab. of Pharmacy Faculty of Ankara University. IR spectra were recorded on a Jasco FT/IR-420 spectrometer (Easton, USA) as potassium bromide discs. ^{1}H NMR spectra were measured with a VARIAN Mercury 400 FT-NMR spectrometer (Palo Alto, CA, USA) in $CDCl_3$. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on Waters Micromass ZQ (Waters Corporation, Milford, MA, USA) by using ESI (+) method. Elementary analyses were determined on a Leco CHNS 932 analyzer (Leco, St. Joseph, MI, USA) and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. For the chromatographic analysis Merck Silica Gel 60 (230–400 mesh ASTM = American Society for Testing and Materials) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA). 2,4-TZD (**I**),[15] 2,4-dichlorothiazole-5-carbaldehyde (**II**),[21] and substituted phenacyl-2,4-TZD (**IVa**, **IVc**, **IVf**,[28] **IVb**,[29] **IVd**[23]) were synthesized according to the literature.

Crystals suitable for X-ray analysis were obtained by recrystallization of compound **VIc-1** from dimethylformamide/isopropanol (1:5) The data collection was performed on a CAD-4 diffractometer employing graphite-monochromated CuK_α radiation ($\lambda = 1.54184$ Å). Three standard reflections were measured every 2 h. The structure was solved by direct methods. The refinement was made with anisotropic temperature factors for all non-hydrogen atoms. The hydrogen atoms were generated geometrically. An empirical Ψ scan absorption correction was applied. Crystallographic data (excluding structure factors) for compound **VIc-1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 626234 Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: depositcdc.cam.ac.uk).

4.2. Synthesis of 2,4-dichlorothiazole-5-carbaldehyde (II)

N,*N*-Dimethylformamide (1.61 g, 0.021 mol) was added dropwise during 5 min to a stirred suspension of 2,4-TZD (**I**) (2.5 g, 0.021 mol) in phosphoryl chloride (19.74 g, 0.129 mol) at 10–20 °C. After the addition the resulting mixture was kept at ambient temperature for 1 h, after which it was heated to 80–90 °C and stirred at this temperature for a further 1 h. The mixture was heated under reflux and stirred at this temperature (~115 °C) until gas evolution ceased (~4 h). After cooling, the reaction mixture was stirred slowly onto ice. The product was extracted with dichloromethane (3× 50 ml) and the extracts were combined, washed successively with aqueous $NaHCO_3$ and water, then dried on anhydrous Na_2SO_4 and distilled off under reduced pressure. The crude product was crystallized from light petroleum (1.6 g, 41.13%). Mp: 48 °C (Ref. 21 mp: 48–49 °C).

4.3. 3-[2-(2,4-Dichloro-phenyl)-2-oxo-ethyl]-thiazolidine-2,4-dione (IVe)

A solution of KOH (0.957 g, 0.017 mol) in CH_3OH (10 ml) was added dropwise to a suspension of 2,4-TZD (2.0 g, 0.017 mol) in 15 ml of CH_3OH. Ten minutes after this addition omega,2,4-trichloroacetophenone (3.816 g, 0.017 mol) was added. The mixture was allowed to stand at room temperature over 5 min then refluxed during 5 h. The crude product was removed by filtration, washed with H_2O and ether. The residue was crystallized from ethanol (yield: 2.12 g, 40.84%; mp 150 °C). ^{1}H NMR ($CDCl_3$): $\delta = 4.07$ (s, 2H, CH_2), 4.94 (s, 2H, N–CH_2), 7.34 (dd, 1H, $J_{5',6'} = 8.40$ Hz, $J_{5',3'} = 2.00$ Hz, 5′-H), 7.46 (d, 1H, $J_{3',5'} = 2.00$ Hz, 3′-H), 7.66 (d, 1H, $J_{6',5'} = 8.40$ Hz, 6′-H). MS (ESI) *m*/*z* (rel intensity): 326.6 (M+22, 65%), Anal. for $C_{11}H_7Cl_2NO_3S$: Calcd C, 43.42; H, 2.30; N, 4.61; S, 10.53. Found C, 43.20; H, 2.30; N, 4.74; S, 10.52.

4.4. Synthesis of compounds VIa–f

A mixture of 2,4-dichloro-thiazole-5-carbaldehyde (**II**) (0.001 mol) and **IVa–f** (0.001 mol) was heated at 130–140 °C in the presence of 0.5 ml acetic acid glacial and sodium acetate (0.001 mol) for 5 h. The reaction mixture was extracted with $CHCl_3$ (3× 50 ml) and the organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography silica gel 60 (230–400 mesh ASTM) using hexane/dichloromethane (1:1) as eluant.

4.4.1. 3-(2-Phenyl-2-oxo-ethyl)-5(2,4-dichloro-thiazole-5yl-methylenyl)-thiazolidine-2,4-dione (VIa). Yield: 51.32%, mp: 168.4 °C, ^{1}H NMR ($CDCl_3$): $\delta = 5.18$ (s, 2 H, CH_2), 7.51–7.55 (m, 2H, 3′, 5′-H), 7.64–7.66 (m, 1H, 4′-H),

7.97–7.99 (m, 2H, 2′, 6′-H), 8.04 (s, 1H, =CH), MS (ESI) *m/z* (rel intensity): 420.9 (M+22, 100%), Anal. for $C_{15}H_8Cl_2N_2O_3S_2$: Calcd C, 45.12; H, 2.02; N, 7.02; S, 16.06. Found C, 44.97; H, 2.12; N, 7.10; S, 16.52.

4.4.2. 3-[2-(4-Fluoro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIb). Yield: 22.91%, mp: 189 °C, ^{1}H NMR ($CDCl_3$): δ = 5.14 (s, 2H, CH_2), 7.19–7.23 (m, 2H, 3′, 5′-H), 8.00–8.03 (m, 2H, 2′, 6′-H), 8.05 (s, 1H, =CH), MS (ESI) *m/z* (rel intensity): 438.9 (M+22, 100%), Anal. for $C_{15}H_7Cl_2FN_2O_3S_2$: Calcd C, 43.18; H, 1.69; N, 6.71; S, 15.37. Found C, 42.77; H, 1.83; N, 6.73; S, 15.35.

4.4.3. (*Z*)3-[2-(4-Chloro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIc-1). Yield: 29.39%, mp: 172.4 °C, ^{1}H NMR ($CDCl_3$): δ = 5.13 (s, 2H, CH_2), 7.51 (d, 2H, 3′, 5′-H), 7.92 (d, 2H, 2′, 6′-H), 8.05 (s, 1H, =CH), MS (ESI) *m/z* (rel intensity): 436.4 (M+H, 70%), Anal. for $C_{15}H_7Cl_3N_2O_3S_2$: Calcd C, 41.54; H, 1.63; N, 6.46; S, 14.79. Found C, 41.32; H, 1.81; N, 6.61; S, 14.69.

4.4.4. (*E*)3-[2-(4-Chloro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIc-2). Yield: 6.3%, mp: 309.5–312 °C, ^{1}H NMR ($CDCl_3$): δ = 5.11 (s, 2H, CH_2), 7.34 (s, 1H, =CH), 7.51 (d, 2H, 3′, 5′-H), 7.92 (d, 2H, 2′, 6′-H). MS (ESI) *m/z* (rel intensity): 436.3 (M+H, 25%), Anal. for $C_{15}H_7Cl_3N_2O_3S_2$: Calcd C, 41.54; H, 1.63; N, 6.46; S, 14.79. Found C, 41.24; H, 1.82; N, 6.44; S, 14.66.

4.4.5. 3-[2-(4-Bromo-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VId). Yield: 34.27%, mp: 177.4 °C, ^{1}H NMR ($CDCl_3$): δ = 5.13 (s, 2H, CH_2), 7.68 (d, 2H, 3′, 5′-H), 7.84 (d, 2H, 2′, 6′-H), 8.04 (s, 1H, =CH), MS (ESI) *m/z* (rel intensity): 500.9 (M+22, 100%), Anal. for $C_{15}H_7BrCl_2N_2O_3S_2$. $0.7H_2O$: Calcd C, 36.69; H, 1.71; N, 5.71; S, 13.05. Found C, 36.52; H, 1.59; N, 5.67; S, 12.91.

4.4.6. (*Z*)3-[2-(2,4-Dichloro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIe-1). Yield: 29.17%, mp: 181.7 °C, ^{1}H NMR ($CDCl_3$): δ = 5.12 (s, 2H, CH_2), 7.39 (dd, 1H, $J_{5',6'}$ = 8.40 Hz, $J_{5',3'}$ = 2.00 Hz, 5′-H), 7.52 (d, 1H, $J_{3',5'}$ = 2.00 Hz, 3′-H), 7.72 (d, 1H, $J_{6',5'}$ = 8.00 Hz, 6′-H), 8.04 (s, 1H, =CH), MS (ESI) *m/z* (rel intensity): 490.9 (M+22, 100%), Anal. for $C_{15}H_6Cl_4N_2O_3S_2$: Calcd C, 38.48; H, 1.29; N, 5.98; S, 13.70. Found C, 38.26; H, 1.40; N, 6.07; S, 13.98.

4.4.7. (*E*)3-[2-(2,4-Dichloro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIe-2). Yield: 4.86%, mp: 179.80 °C, ^{1}H NMR ($CDCl_3$): δ = 5.10 (s, 2H, CH_2), 7.33 (s, 1H, =CH), 7.39 (dd, 1H, $J_{5',6'}$ = 8.40 Hz, $J_{5',3'}$ = 2.00 Hz, 5′-H), 7.52 (d, 1H, $J_{3',5'}$ = 2.00 Hz, 3′-H), 7.72 (d, 1H, $J_{6',5'}$ = 8.40 Hz, 6′-H), MS (ESI) *m/z* (rel intensity): 490.9 (M+22, 21%), 468.9 (M+H, 21%), Anal. for $C_{15}H_6Cl_4N_2O_3S_2$: Calcd C, 38.48; H, 1.29; N, 5.98; S, 13.70. Found C, 38.95; H, 1.39; N, 6.08; S, 13.31.

4.4.8. 3-[2-(4-Nitro-phenyl)-2-oxo-ethyl]-5-(2,4-dichloro-thiazole-5-yl-methylenyl)-thiazolidine-2,4-dione (VIf). Yield: 64.56%, mp: 159 °C, ^{1}H NMR ($CDCl_3$): δ = 5.19 (s, 2H, CH_2), 8.06 (s, 1H, =CH), 8.16 (d, 2H, 3′, 5′-H), 8.39 (d, 2H, 2′, 6′-H), MS (ESI) *m/z* (rel intensity): 465.99 (M+22, 100%), Anal. for $C_{15}H_7Cl_2N_3O_5S_2$ $0.2H_2O$: Calcd C, 40.21; H, 1.65; N, 9.38; S, 14.29. Found C, 39.83; H, 1.73; N, 9.23; S, 14.19.

4.5. Microbiology

For the antibacterial and antimycotic assays, the compounds were dissolved in dimethylformamide/propyleneglycol (0.5:9.5). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/ml concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the twofold serial dilution technique.[27] A control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions used in our experiments and found inactive in the culture medium. All the compounds were tested for their in vitro growth inhibitory activity against different bacteria and the yeasts Candida albicans ATCC 10145, Origins of bacterial strains are *S. aureus* ATCC 29253, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (MRSA) (isolate) as Gram-positive and *E. coli* ATCC 23556 as Gram-negative bacteria. ATCC strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of Faculty of Pharmacy of Ankara University. Ampicillin, ciprofloxacin, and miconazole were used as control drugs. The data on the antimicrobial activity of the compounds and the control drugs as MIC (μg/ml) values are given in Table 1.

5. Antibacterial and antifungal assays

The cultures were obtained from Mueller–Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 ± 1 °C. *C. albicans* were maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 1 °C. Testing was carried out in Mueller–Hinton broth and Sabouraud dextrose broth (Difco) at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^5 CFU/ml for the antibacterial assay and 10^4 CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37 ± 1 °C and after incubation for 48 h at 25 ± 1 °C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC expressed in mg/ml. Every experiment in the antibacterial and antifungal assays was replicated twice.

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