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Synthesis and antimicrobial evaluation of some 3-(substituted phenacyl)-5-[4′-(4*H*-4-oxo-1-benzopyran-2-yl)-benzylidene]-2,4-thiazolidinediones

Meral Tunçbilek [a,*], Nurten Altanlar [b]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey*
[b] *Department of Microbiology, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey*

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Abstract

Synthesis and in vitro antimicrobial activity of some 3-(substituted phenacyl)-5-[4′(4*H*-4-oxo-1-benzopyran-2-yl)-benzylidene]-2,4-thiazolidinedione derivatives are described. These products were synthesized by the Knoevenagel reaction from 4′-flavone carboxaldehyde and 3-substituted phenacyl-2,4-thiazolidinediones.

Keywords: Flavonylthiazolidinediones; Antimicrobial activity

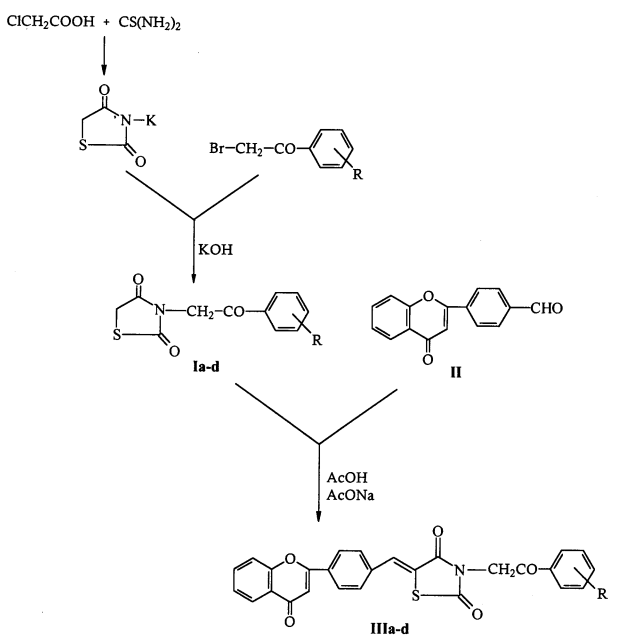
1. Introduction

Considerable interest has been focused on thiazolidinedione derivatives which have been shown to possess a broad spectrum of biological activities. The most important of these are antibacterial, antifungal [1–3], antidiabetic [4], cardiotonic [5], anti-oedematous and analgesic [6], anticonvulsant [7], cyclooxygenase and lipoxygenase inhibitory [8] activities. On the other hand, flavonoids possess spasmolytic [9], capillary resistance activity [10], antidiabetic [11], coronary dilatory [12] and antibacterial [13] effects.

Based on the above-mentioned facts, we synthesized a series of compounds containing the thiazolidinedione moiety and flavone nucleus in one frame in order to study their antimicrobial activity.

2. Chemistry

The synthetic routes followed for the preparation of the new compounds are illustrated in Scheme 1. Compounds **Ia–b** [14], **Ic–d** [15] and **II** [16] were synthesized according to the literature. The linkage between the flavone and 2,4-thiazolidinedione moieties were obtained by Knoevenagel condensation of the 4′-flavon carboxaldehyde (**II**) and 2,4-thiazolidinedione ring (**Ia–d**). Sodium acetate/glacial acetic acid were gener-



Scheme 1. Preparation of the compounds **IIIa–d**.

* Corresponding author.

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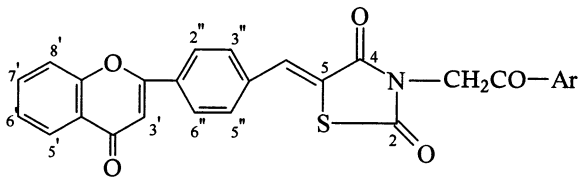
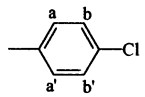
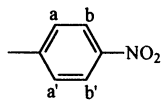
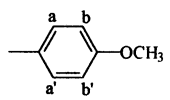
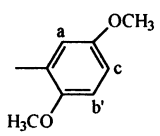
Table 1
Some physicochemical properties of compounds **IIIa–d**



Comp.	Ar	Yield (%)	Melting point (°C)	Formula	Analysis	IR (cm^{-1})
IIIa		66	257	$C_{27}H_{16}ClNO_5S$	C, H, N	1755 (C^4=O), 1693 (C^2=O), 1649 (γ-pyron C=O), 1590 (C=C)
IIIb		30	287	$C_{27}H_{16}N_2O_7S$	C, H, N	1756 (C^4=O), 1696 (C^2=O), 1649 (γ-pyron C=O), 1598 (C=C)
IIIc		59	252	$C_{28}H_{19}NO_6S$	C, H, N	1752 (C^4=O), 1682 (C^2=O), 1643 (γ-pyron C=O), 1597 (C=C)
IIId		45	270	$C_{29}H_{21}NO_7S$	C, H, N	1732 (C^4=O), 1676 (C^2=O), 1644 (γ-pyron C=O), 1599 (C=C)

ally used as reagents in this condensation. Some physicochemical properties of the compounds are given in Table 1. The structures assigned to new products were confirmed by IR, ^{1}H NMR and mass spectral data.

^{1}H NMR and mass spectra values of the compounds are listed in Section 3. All spectral data are in accordance with assumed structures. In ^{1}H NMR spectra, C-3, C-5, C-6, C-7 and C-8 protons of 4*H*-benzopyran ring and flavone B ring protons were seen between 7.18–8.14 ppm, CH_2N protons were observed at 5.09–5.47 ppm as a singlet. Mass spectrometric analyses were performed by the electron impact (EI) method. All the fragments appeared at the expected m/z values.

Substituted 5-arylidene-2,4-thiazolidinediones are theoretically able to exist in the *Z* and *E* configurations. However, in the reactions between unsubstituted imidazolidinediones and benzaldehyde in acidic medium, the main product was the *Z* isomer [17]. Moreover, the coupled ^{13}C NMR study, given the vicinal coupling constant of the ethylenic proton and the carbonyl group, shows that arylidene thiazolidinediones were also in the *Z* configuration [18,19].

3. Experimental

Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrometer as potassium bromide discs. ^{1}H NMR spectra were measured with a Bruker GmbH DPX-400, 400 MHz instrument using TMS internal standard and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. EIMS were obtained with a VG Platform II, Micromass spectrometer with ionization energy maintained at 70 eV. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument, and were within $\pm 0.4\%$ of the theoretical values. Instrumental analyses were performed at the Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara, Turkey). The chemical reagents used in the synthesis were purchased from E. Merck and Aldrich. RSKK strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, Turkey.

3.1. General synthesis of compounds IIIa–d

3-(Substituted phenacyl)-2,4-thiazolidinedione (**Ia**–**d**) (1 mmol) and CH_3COONa (0.25 g) were added to a solution of 4′-flavone carboxaldehyde (**II**) (1.2 mmol) in glacial CH_3COOH (3 ml). The reaction mixture was heated to 140–150°C for a period of 4–6 h. The resulting precipitate was filtered, washed with H_2O and then with acetone (Table 1).

3.1.1. 3-(4-Chlorophenacyl)-5-[4′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (IIIa)

^{1}H NMR (DMSO-d_6): δ 5.37 (s, 2H, CH_2N), 7.18 (s, 1H, 3′′-H), 7.54 (ddd, 1H, $J_{6',5'} = J_{6',7'} = 7.66$ Hz, 6-′H), 7.69 (d, 2H, b, b′-H), 7.82–7.90 (m, 4H, 3′′,5′′,7′,8′-H), 8.07–8.14 (m, 4H, a, a′, =CH, 5′-H), 8.31 (d, 2H, $J_{2'',3''} = J_{6'',5''} = 8.23$ Hz, 2′′,6′′-H). EIMS [m/z (rel. int.%)]: 206 (2.9), 158 (2.6), 139 (70), 111 (70.6), 102 (10), 92 (75.9), 74 (100), 64 (20.6), 63 (51).

3.1.2. 3-(4-Nitrophenacyl)-5-[4′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (IIIb)

^{1}H NMR (DMSO-d_6): δ 5.47 (s, 2H, CH_2N), 7.18 (s, 1H, 3′′-H), 7.54 (ddd, 1H, $J_{6',5'} = J_{6',7'} = 7.64$ Hz, 6′H), 7.82–7.90 (m, 4H, 3′′,5′′,7′,8′-H), 8.08 (d, 1H, $J_{5',6'} =$ 7.74 Hz, 5′-H), 8.12 (s, 1H, =CH), 8.31–8.36 (m, 4H, a, a′, b, b′-H), 8.41 (d, 2H, $J_{2'',3''} = J_{6'',5''} = 8.84$ Hz, 2′′,6′′-H). EIMS [m/z (rel. int.%)]: 278 (3.5), 176 (8.2), 150 (20), 120 (28.2), 92 (100), 76 (75.8), 64 (50.9), 63 (87.3), 56 (7.9).

3.1.3. 3-(4-Methoxyphenacyl)-5-[4′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (IIIc)

^{1}H NMR (DMSO-d_6): δ 3.90 (s, 3H, OCH_3), 5.29 (s, 2H, CH_2N), 7.13 (d, 2H, $J_{b,a} = J_{b',a'} = 8.89$ Hz, b, b′-H), 7.18 (s, 1H, 3′′-H), 7.54 (ddd, 1H, $J_{6',5'} = J_{6',7'} = 7.95$ Hz, $J_{6',8'} = 1.23$ Hz, 6′-H), 7.82–7.90 (m, 4H, 3′′,5′′,7′,8′-H), 8.07–8.11 (m, 4H, =CH, 5′, a, a′-H), 8.30–8.33 (d, 2H, $J_{2'',3''} = J_{6'',5''} = 8.49$ Hz, 2′′,6′′-H). EIMS [m/z (rel. int.%)]: 176 (19.4), 135 (22.9), 120 (6.5), 102 (5.3), 92 (100), 64 (23.5), 63 (55.6), 56 (84.4).

3.1.4. 3-(2,5-Dimethoxyphenacyl)-5-[4′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (IIId)

^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H, 5-OCH_3), 3.97 (s, 3H, 2-OCH_3), 5.09 (s, 2H, CH_2N), 7.18 (s, 1H, 3′-H), 7.27–7.29 (m, 3H, 3′, 4′, 6′-H), 7.54 (ddd, 1H, $J_{6',5'} = J_{6',7'} = 6.77$ Hz, 6′-H), 7.82–7.90 (m, 4H, 3′′,5′′,7′,8′-H), 8.08–8.10 (m, 2H, =CH, 5′-H), 8.31 (d, 2H, $J_{2'',3''} = J_{6'',5''} = 8.45$ Hz, 2′′,6′′-H). EIMS [m/z (rel. int.%)]: 278 (2.4), 221 (4.2), 165 (56.4), 135 (11.5), 120 (13.9), 107 (50.3), 92 (97.6), 63 (100), 56 (30.0).

4. Antimicrobial activity

The in vitro antimicrobial activity of the compounds was tested by the tube dilution technique [20]. Test and reference compounds (Ampicillin trihydrate, Fluconazole and Ketoconazol) were dissolved in 12.5% DMSO, at concentrations of 50 μg/ml, further dilutions of the compounds and standards in the test medium were prepared at the required concentrations of 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/ml. The final inoculum size was 10^5 CFU/ml. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antimicrobial activity against any of the test microorganisms.

All the compounds were tested for their in vitro growth inhibitory activity against *Staphylococcus aureus* ATCC 250 as Gram-positive and *Escherichia coli* RSKK 313 as Gram-negative bacteria and a fungus, *Candida albicans* RSKK 628. MIC values of new and reference compounds are presented in Table 2.

4.1. Antibacterial activity assay

The cultures were obtained in Mueller–Hinton Broth (Difco) for all the bacteria after 18–24 h of incubation at 37 ± 1°C. Testing was carried out in Mueller–Hinton Broth at pH 7.4 and a two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 18–24 h at 37 ± 1°C, the last tube with no microorganism growth was recorded to represent MIC expressed in μg/ml.

4.2. Antifungal activity assay

The yeast *C. albicans* was maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1°C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the two-fold dilution tech-

Table 2
Antimicrobial activities [a] of compounds **IIIa–d**

Comp.	*C. albicans*	*S. aureus*	*E. coli*
IIIa	12.5	12.5	[c]
IIIb	12.5	12.5	[c]
IIIc	25	25	[c]
IIId	25	25	[c]
Ketoconazole	6.25	[b]	[b]
Fluconazole	12.5	[b]	[b]
Ampicillin	[b]	0.78	6.25

[a] MIC.
[b] Not tested.
[c] No activity.

nique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1°C, the last tube with no yeast growth was recorded to represent MIC expressed in μg/ml.

5. Results and discussion

Compounds **IIIa–d** were evaluated for their in vitro antimicrobial activity against *C. albicans*, *S. aureus* and *E. coli* by tube dilution technique. Their antibacterial and antifungal activities were determined as MIC values. All the investigated compounds showed only a moderate activity against *S. aureus*. It is noteworthy that compounds **IIIa–b** containing *p*-chloro or *p*-nitro substituents, showed an activity comparable to that of Fluconazole against *C. albicans*. None of the compounds exhibited activity towards *E. coli*.

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