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ORIGINAL ARTICLE

Synthesis, characterization and biological activity of 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl) propanoic acid derivatives

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2,4-Thiazolidinedione;
X-ray analysis;
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Antimicrobial activity

Abstract A series of six novel 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acids and six corresponding methyl esters were synthesized. All compounds were characterized by melting points, elemental analysis, FT-IR, ^{1}H and ^{13}C NMR spectroscopy. Crystal structure of methyl-2-(5-(4-methoxybenzylidene)-2,4–dioxotetrahydrothiazole-3-yl)propionate was confirmed by X-ray analysis. The antiproliferative activity of all synthesized compounds against human colon cancer, breast cancer and myelogenous leukemia cell lines, *i.e.* HCT-116, MDA-231 and K562, respectively, was evaluated. The results indicate that antiproliferative activity of the synthesized esters is better than the activity of the corresponding acids. All synthesized compounds showed significant antiproliferative effects against HCT116 cells in all tested concentrations (0.01–100 μM). Moreover, *in vitro* antimicrobial activity against a wide range of tested microorganisms was examined.

1. Introduction

Drugs with two or more mechanisms of action and with potential to target on different diseases may in some cases offer more therapeutic benefit than drugs that target one disease only. Moreover, such multiple mechanism drugs may exhibit a more favorable side effect profile than a polypharmaceutical combination of several drugs that individually target the same disease (Youdim et al., 2007; Petzer et al., 2009).

2,4-Thiazolidinediones (TZDs), also known as glitazones, are a class of antidiabetic drugs. Their mechanisms of action are mediated through activation of the peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily of ligand-dependent transcription factors predominantly expressed in adipocytes, but also in other normal and transformed cells (Rocchi and Auverrx,

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1999). There are several studies reporting that TZDs have acquired much importance because of their diverse pharmaceutical applications such as antiproliferative (Salamone et al., 2012), bactericidal (Bozdag-Dundar et al., 2008), pesticidal (Eun et al., 2007), fungicidal (Mori et al., 2008), insecticidal (Sahu et al., 2007), antihyperglycemic (Lee et al., 2005), anticonvulsant (Dwivedi et al., 1972), tuberculostatic (Verma and Saraf, 2008), anti-inflammatory (Ceriello, 2008), etc. For the synthesis of TZD derivatives with potential biological activity, the most interesting centers of reactivity are C(5) and N(3) (Fig. 1).

On the other hand, propanoic acid derivatives (ibuprofen, naproxen, ketoprofen, etc.) belong to the class of non-steroidal anti-inflammatory drugs (NSAIDs). Their therapeutic effects result from a selective inhibition of the enzyme cyclooxygenase-2 (COX-2), which is largely responsible for the production of prostaglandins in most pathological states (Talley et al., 2000). Numerous experimental, epidemiologic and clinical studies suggest that NSAIDs, particularly the highly selective COX-2 inhibitors, are potential anticancer agents (Cha and DuBois, 2007; Thun et al., 2002; Lanas, 2009). Beneficial effects of NSAIDs can be explained by an independent mechanism of action in relation to the COX-inhibition pathway (Tegeder et al., 2001), which is mediated through modulation of activity of various intracellular kinases including PPARγ inducing apoptosis (Liou et al., 2007; Baek et al., 2005; Wahl et al., 1998; Ho et al., 2003).

Bearing in the mind that TZDs and NSAIDs could induce PPARγ activation which participate in cell apoptosis, synthesis of combined molecules containing both drugs is very promising regarding potential cumulative antiproliferative effects.

In the course of identifying various compounds that may serve as leads for designing novel antiproliferative or antimicrobial agents, in this study, six novel 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acids and six corresponding methyl esters were synthesized and characterized (Fig 1.). All compounds were tested *in vitro* against human colon cancer, breast cancer and myelogenous leukemia cell lines, *i.e.* HCT-116, MDA-231, and K562, respectively, by MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Moreover, their *in vitro* antimicrobial activities against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and clinical isolates of *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *S. aureus* and *Candida albicans* were evaluated.

2. Results and discussion

2.1. Chemistry

Considering the role of various 2,4-thiazolidinediones and propanoic acid derivatives in biological activity, synthesis of 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acid derivatives was reported. Six novel 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acids (**7**–**12**) were obtained from the corresponding methyl esters (**1**–**6**).

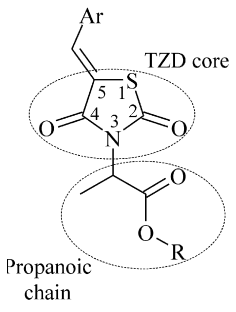
The synthetic route of the compounds (**1**–**12**) is outlined in Scheme 1. The 2,4-thiazolidinedione was prepared by cyclization of equimolar quantities of chloroacetic acid and thiourea by published procedures (Andreani et al., 1993). Corresponding aromatic amines were subjected to the Knoevenagel condensation with 2,4-thiazolidinedione in the presence of a catalytic amount of morpholine and acetic acid to afford 5-arylidene-2,4-thiazolidinediones (Popov-Pergal et al., 1991), which were further alkylated in position 3 using 2-bromopropionate (Suzuki et al., 2004). Thus obtained esters **1**–**6** were undergoing the hydrolysis catalyzed by mineral acid in order to prepare corresponding acids (**7**–**12**).

All synthesized compounds (**1**–**12**) were purified by recrystallization from DMF and characterized by spectral (FT-IR, ^{1}H and ^{13}C NMR) and elemental analysis. The results of spectral analysis indicated that the compounds are pure.

2.2. Crystal structure of compound 2

The colorless prismatic single crystals of **2** were obtained from an EtOH solution by slow evaporation in a freezer. The first crystals appeared within two weeks, but the single crystals of a suitable size were filtered off after about one month.

Selected crystal data and refinement results are listed in Table 1, the molecular structure with atomic labeling scheme and crystal packing are depicted in Figs. 2 and 3, respectively.



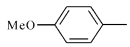
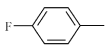
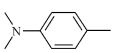
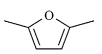
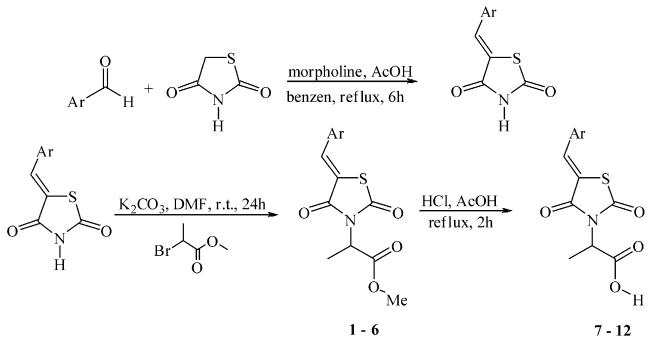
Ar–	Compound	
	Ester, R = Me	**Acid,** R = H
	**1**	**7**
	**2**	**8**
	**3**	**9**
	**4**	**10**
	**5**	**11**
	**6**	**12**

Figure 1 Structure of 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acid derivatives.



Scheme 1 Preparation of 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acid derivatives (**1**–**12**).

Table 1 Crystal data and refinement results for 2.

Chemical formula	$C_{15}H_{15}NO_5S$
Formula weight	321.34
Wavelength (Å)	0.71073
Crystal system, space group	Orthorhombic, *Pbcn*
a (Å)	16.7715(4)
b (Å)	7.5358(8)
c (Å)	24.9575(13)
V (Å^3)	3154.3(4)
Z	8
Calculated density (Mg m^{-3})	1.353
F(000)	1344
Crystal size (mm^3)	$0.17 \times 0.08 \times 0.07$
Absorption coefficient (mm^{-1})	0.227
Limiting indices	$-20 \leqslant h \leqslant 20$, $-9 \leqslant k \leqslant 7$, $-30 \leqslant l \leqslant 30$
Reflections collected/unique	19,033/2981 [*R*(int) = 0.0365]
Theta range for data collection (°)	2.03 to 25.68
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2981/0/207
Goodness of fit on F^2	1.149
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0530$, $wR_2 = 0.1310$
R indices (all data)	$R_1 = 0.0614$, $wR_2 = 0.1378$
Largest diff. peak and hole (e Å^{-3})	0.165 and −0.194

The bond distances and angles of the TZD ring in **2** are as expected for such type of compounds (Sun et al., 2008). The TZD and Ar rings are almost coplanar with dihedral angle of only 3.77°. In this way, an extended core with electrons delocalized over TZD and Ar rings, as well as over C5–C8 and C8–C9 bonds is found to exist. The same feature could be expected for all synthesized compounds (**1**–**12**). The angle between C12/C14/O4/O5 plane and TZD ring is 68.93°. The value of torsion angle O4–C14–O5–C15 in the propanoic chain is only 0.54°, which could be the reason of favorable biological properties.

The molecules are held together by van der Waals forces forming wave-like pseudo-chains extending along *c*-axis (Fig. 3). The chains are further packed in 3D network *via* face to face π–π interactions. Double π–π interactions exist between the adjacent molecules involving Ar, with the shortest C3–C7 distance 3.384(4) Å, and TZD rings, with the shortest S1–C9 distance 3.576(2) Å.

2.3. Biological studies

2.3.1. Non-specific cytotoxic activity

The first step for biological characterization of investigated compounds was non-specific cytotoxic evaluation. Examination of cytotoxic effect (non-specific cells killing) to viability of unstimulated and LPS stimulated rat peritoneal macrophages by MTT assay was conducted. This part of the study demonstrated that the investigated compounds did not exhibit the cytotoxic effect to peritoneal macrophages (data not shown), which are well-known cells applied for this type of non-specific cytotoxic investigation.

2.3.2. Antiproliferative activity

The antiproliferative action of substances **1**–**12** was tested against malignant cell lines: human colon cancer HCT-116, human breast cancer MDA-231 and human myelogenous leukemia K562 cells in the 0.01–100 μM range of concentration.

With exception of **1** and **8** in the lowest concentration (0.01 μM), all investigated compounds exhibited significant antiproliferative effects against human colon cancer HCT116 cells in all tested concentrations (Table 2). Additionally, the correlation between the dose of the drug and corresponding inhibition of cell proliferation was analyzed. In all cases, the analysis of corresponding ester/acid pairs showed that coefficients of correlation (*R*) for ester compared to the corresponding acid is higher (for all investigated compounds *R* values were higher than 0.90). Pair **1**/**7** expressed exponential correlations, which indicate dose-dependent effects of these compounds. Further, pairs **4**/**10**, **5**/**11** and **6**/**12** expressed linear correlations, which indicate dose-dependent effects of these compounds, as well. On the other hand, for pairs **2**/**8** and **3**/**9** squared correlations were observed, which could suggest a different mechanism of action of these compounds at higher and lower concentrations. For pairs **2**/**8** and **3**/**9,** it could be assumed that the mechanism in high doses was similar to the mechanism that other investigated drugs expressed, but in low-

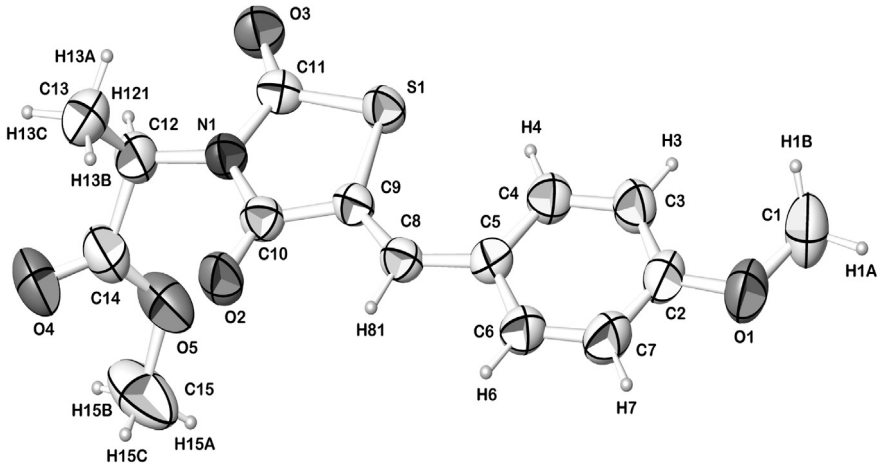


Figure 2 Molecular structure of **2** with atomic labeling scheme. Displacement ellipsoids are drawn at the 50% probability level.

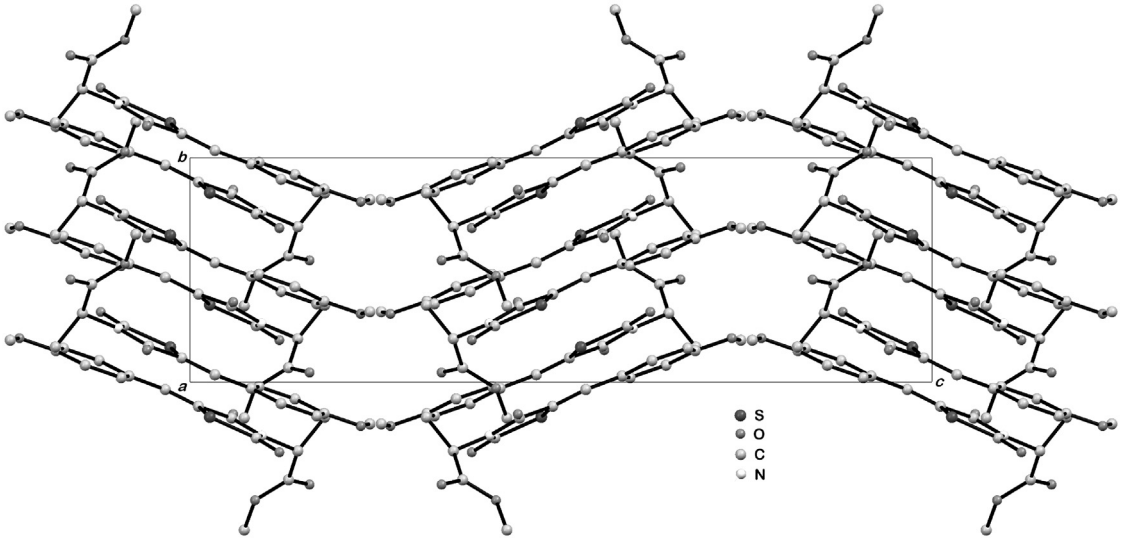


Figure 3 Crystal packing of **2**, projection along *a*-axis (*c*-axis is horizontal).

er concentrations these drugs use another mechanism to prevent proliferation of investigated tumor cell lines.

A satisfactory inhibitory activity of **1**–**12** to the proliferation of the second tumor cell line, MDA-231, was obtained only at the highest concentration (100 μM). As shown in Fig. 4, the esters **1**–**3**, **5,** and **6** exhibited a significant antiproliferative activity toward MDA231 cells, which is not true for the corresponding acids (100 μM). This inhibition was lower compared to the inhibitory effects on HCT-116 cells. These results are consistent with the previous finding which showed higher R for ester when analyzed HCT-116 cell survival in the presence of investigated compounds.

The third cell line used for antiproliferative investigation of synthesized TZD esters and acids was human myelogenous leukemia K562. The results of this analysis showed antiproliferative activity of the following compounds: **1** (10%), **5** (12%), **6** (12%), **9** (3%), and **12** (7%). As in previous experiments with other cell lines, esters (**1**, **5**, **6**) revealed a better activity for inhibition of K562 cells proliferation than the acids (**9**, **12**).

It could be concluded that all synthesized compounds demonstrate a satisfactory inhibitory activity to the proliferation of different investigated tumor cell lines without non-specific cytotoxic effects. Moreover, these results indicate better properties of esters for antiproliferative activity of investigated compounds than the corresponding acids and the need for further investigation about detailed specific mechanisms of ester activity in this and other experimental systems.

2.3.3. Antimicrobial activity

The analyzed compounds did not show antimicrobial activity to tested microorganisms including *C. albicans*, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Bacillus subtilis*, *Klebsiela pneumones*, and *E. faecalis*.

3. Material and methods

3.1. Reagents and analysis

The starting materials were obtained from Aldrich and Fluka, and were used without further purification. The molecular structures and purities of the synthesized **1**–**12** were confirmed

Table 2 The antiproliferative effects of **1**–**12** on HCT-116 cell line.

No./Conc.	Inhibition of proliferation of HCT116 cells (%)					
	100 μM	50 μM	10 μM	1 μM	0.1 μM	0.01 μM
1	16.69 ± 2.89*	15.24 ± 2.35*	11.27 ± 4.69*	10.85 ± 4.02*	8.66 ± 2.59*	4.80 ± 0.50
2	16.90 ± 1.88*	17.97 ± 6.16*	9.67 ± 2.70*	10.32 ± 2.41*	14.65 ± 2.49*	11.15 ± 3.58*
3	15.18 ± 1.48*	16.79 ± 1.69*	13.35 ± 2.66*	14.12 ± 1.97*	9.79 ± 3.80*	11.51 ± 3.48*
4	36.06 ± 1.95*	25.86 ± 3.46*	14.83 ± 5.54*	19.45 ± 3.24*	17.02 ± 2.78*	13.94 ± 0.67*
5	32.80 ± 1.25*	21.12 ± 5.46*	15.60 ± 2.11*	12.69 ± 3.70*	11.98 ± 2.00*	11.80 ± 3.73*
6	27.76 ± 7.76*	12.34 ± 2.20*	11.15 ± 4.26*	12.63 ± 2.16*	9.02 ± 4.47*	10.79 ± 2.85*
7	17.32 ± 3.82*	21.35 ± 2.14*	14.53 ± 6.05*	15.30 ± 4.27*	13.52 ± 2.27*	9.55 ± 3.34*
8	18.27 ± 1.78*	17.38 ± 4.11*	6.47 ± 1.79*	7.83 ± 1.85*	11.33 ± 4.47*	6.76 ± 2.26
9	18.86 ± 7.18*	19.81 ± 6.27*	12.75 ± 5.05*	17.91 ± 0.54*	12.10 ± 2.74*	13.05 ± 4.58*
10	25.74 ± 0.54*	28.05 ± 4.52*	20.11 ± 6.03*	14.89 ± 2.29*	13.94 ± 4.92*	16.31 ± 4.22*
11	25.62 ± 0.47*	18.86 ± 4.68*	16.79 ± 4.52*	23.13 ± 4.93*	18.92 ± 3.47*	14.23 ± 3.02*
12	8.24 ± 3.60*	7.83 ± 0.62*	4.03 ± 1.87*	11.98 ± 4.04*	12.81 ± 2.05*	12.63 ± 2.02*

Data presented as mean ± standard deviation (SD).
* $p < 0.05$ vs non-treated cells.

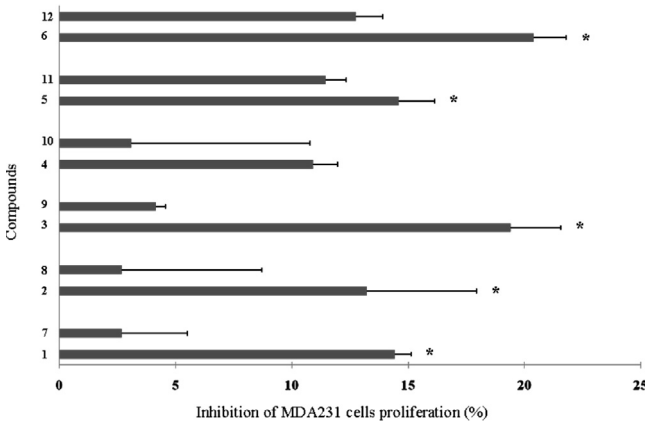


Figure 4 The effect of different TZDs at the concentration of 100 μM to inhibition of MDA231 cell proliferation ($^{*}p < 0.05$ vs non-treated cells).

by melting points, elemental analysis, FT-IR, ^{1}H and ^{13}C NMR spectroscopy and X-ray crystal structure analysis. All melting points were uncorrected. Elemental analysis was realized using an Elemental Vario EL III microanalyzer. The FT-IR spectra were recorded on a Bomem MB 100 spectrophotometer in the form of the KBr pallets. The ^{1}H and ^{13}C NMR spectral measurements were performed on a Bruker AC 250 spectrometer at 200 MHz for the ^{1}H NMR and 50 MHz for the ^{13}C NMR spectra. The spectra were recorded at room temperature in DMSO-d_6. The chemical shifts were expressed in ppm values referred to TMS ($\delta_H = 0$ ppm) in the ^{1}H NMR spectra and the residual solvent signal ($\delta_C = 39.5$ ppm) in the ^{13}C NMR spectra. X-ray single-crystal diffraction data were collected using an Oxford Gemini S diffractometer equipped with a charge-coupled device (CCD) detector at 293 K. Mo Kα radiation ($\lambda = 0.71073$ Å) was used and a multi-scan correction for absorption was applied. The structure of **2** was solved by direct methods (SIR92 (Altomare et al., 1994)) and refined on F^2 by full-matrix least-squares (SHELXL97 (Sheldrick, 2008) and WinGX (Farrugia, 1999)). All nonhydrogen atoms were refined anisotropically. Hydrogen atoms were placed at geometrically calculated positions and refined using the riding model with fixed C–H distances. Positions of hydrogen atoms H81 and H121 were derived from ΔF maps and these atoms were refined isotropically.

3.2. Preparation of 5-arylidene-2,4-dioxotetrahydrothiazoles

A mixture of corresponding aromatic aldehyde (0.025 mol), 2,4-dioxotetrahydrothiazole (0.025 mol), benzene (15 mL), glacial AcOH (0.5 mL) and morpholine (1 mL) was refluxed for 4 h in a Dean–Stark apparatus. After cooling to room temperature products were crystallized, filtered and recrystallized from DMF providing pure compounds (Popov-Pergal et al., 1991).

*3.3. Preparation of methyl-2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propionates (**1**–**6**)*

The appropriate 5-arylidene-2,4-dioxotetrahydrothiazole (0.010 mol) was dissolved in 60 mL of DMF and K_2CO_3 (0.041 mol) was added. 2-Bromopropionate (0.011 mol) was then added and the reaction mixture was stirred overnight. The reaction mixture was poured into three volumes of water and extracted with ethyl acetate. The ethyl acetate extracts were washed with 5% NaOH and water, and then dried over $MgSO_4$. The solvent was removed and the resulting solid was recrystallized from EtOH. The melting points, FT-IR, ^{1}H and ^{13}C NMR spectra of compounds **1**–**6** are in agreement with literature data (Popov-Pergal et al., 2003) and undoubtedly corroborate their structures.

*3.4. Preparation of 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propionic acids (**7**–**12**)*

A mixture of corresponding methyl-2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propionate (0.010 mol), glacial AcOH (40 mL) and conc. HCl (10 mL) was refluxed for 2 h. After evaporation in vacuo, the residue was refluxed again with glacial AcOH (40 mL) and conc. HCl (10 mL) for 2 h. After evaporation to dryness in vacuo, the crude solid was washed with H_2O and recrystallized from EtOH providing pure products (**7**–**12**).

3.5. Characterization

*3.5.1. 2-(5-Benzylidene-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**7**)*

White solid; yield: 54%; m.p.: 177–178 °C; *Anal.* Calcd. for $C_{13}H_{11}NO_4S$ (%): C, 56.31; H, 4.00; N, 5.05. Found (%): C, 56.36; H, 3.98; N, 5.04; FT-IR: *v* (KBr): 3421, 2930, 1713, 1691, 1607, 1370, 1179, 1127 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.95 (s, 1H, –CH═), 7.65–7.51 (m, 5H, C_6H_5), 5.02 (q, J = 6.8 Hz, 1H, CH_3CHCO_2H), 1.51 (d, J = 7.4 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.4 (CO_2H), 167.0 (C═O), 165.2 (C═O), 134.1 (C_6H_5), 133.1 (–CH═), 131.1 (C_6H_5), 130.5 (C_6H_5), 129.7 (C_6H_5), 120.8 (═C–S), 50.6 (CH_3CHCO_2H), 14.2 (CH_3CHCO_2H) ppm.

*3.5.2. 2-(5-(4-Methoxybenzylidene)-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**8**)*

Pale yellow solid; yield: 51%; m.p.: 201–203 °C; *Anal.* Calcd. for $C_{14}H_{13}NO_5S$ (%): C, 54.72; H, 4.26; N, 4.56. Found (%): C, 54.69; H, 4.23; N, 4.58; FT-IR: *v* (KBr): 3448, 2925, 1736, 1683, 1596, 1512, 1360, 1257, 1178 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 7,90 (s, 1H, –CH═), 7.58 (d, J = 8.4 Hz, 2H, C_6H_4), 7.10 (d, J = 8.4 Hz, 2H, C_6H_4), 5.14 (q, J = 6.8 Hz, 1H, CH_3CHCO_2H), 3.82 (s, 3H, $CH_3OC_6H_5$), 1.51 (d, J = 6.8 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.5 (CO_2H), 167.1 (C═O), 165.4 (C═O), 161.6 (C_6H_5), 134.1 (–CH═), 132.7 (C_6H_5), 125.6 (C_6H_5), 117.6 (═C–S), 115.3 (C_6H_5), 55.8 ($CH_3OC_6H_5$), 50.6 (CH_3CHCO_2H), 14.2 (CH_3CHCO_2H) ppm.

*3.5.3. 2-(5-(4-Fluorobenzylidene)-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**9**)*

Pale yellow solid; yield: 55%; m.p.: 200–201 °C; *Anal.* Calcd. for $C_{13}H_{10}FNO_4S$ (%): C, 52.88; H, 3.41; N, 4.74. Found (%): C, 52.93; H, 3.40; N, 4.73; FT-IR: *v* (KBr): 3432, 2927, 1719, 1679, 1596, 1376, 1242, 1176 cm^{-1}; ^{1}H NMR

(200 MHz, DMSO-d_6): δ = 7.97 (s, 1H, –CH═), 7.69 (d, J = 8.4 Hz, 2H, C6H4), 7.37 (d, J = 8.6 Hz, 2H, C6H4), 5.02 (q, J = 7.2 Hz, 1H, CH_3CHCO_2H), 1.51 (d, J = 6.8 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.4 (CO_2H), 167.0 (C═O), 165.2 (C═O), 133.1 (–CH═), 133.0 (C_6H_5), 117.1 (C_6H_5), 116.9 (C_6H_5), 120.7 (═C–S), 50.7 (CH_3CHCO_2H), 14.2 (CH_3CHCO_2H) ppm.

3.5.4. 2-(5-(4-Dimethylaminobenzylidene)-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**10**)

Yellow solid; yield: 58%; m.p.: 233–235 °C; *Anal*. Calcd. for $C_{15}H_{16}N_2O_4S$ (%): C, 56.24; H, 5.03; N, 8.74. Found (%): C, 56.23; H, 5.00; N, 8.75; FT-IR: ν (KBr): 3441, 2922, 1742, 1716, 1683, 1584, 1531, 1363, 1304, 1194 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.79 (s, 1H, –CH═), 7.44 (d, J = 8.0 Hz, 2H, C_6H4), 6.80 (d, J = 8.4 Hz, 2H, C_6H_4), 4.98 (q, J = 7.4 Hz, 1H, CH_3CHCO_2H), 3.00 (s, 6H, $(CH_3)_2N$), 1.49 (d, J = 6.8 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.6 (CO_2H), 167.3 (C═O), 165.4 (C═O), 151.9 (C_6H_4), 135.1 (–CH═), 132.8 (C_6H_4), 120.0 (═C–S), 112.8 (C_6H_4), 112.4 (C_6H_4), 50.3 (CH_3CHCO_2H), 39.8 ($(CH_3)_2N$), 14.3 (CH_3CHCO_2H) ppm.

3.5.5. 2-(5-(5-Methyl-2-furfurylidene)-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**11**)

Yellow solid; yield: 53%; m.p.: 170–172 °C; *Anal*. Calcd. for $C_{12}H_{11}NO_5S$ (%): C, 51.24; H, 3.94; N, 4.98. Found (%): C, 51.20; H, 3.93; N, 5.00; FT-IR: ν (KBr): 3431, 2925, 1725, 1681, 1618, 1567, 1515, 1368, 1235, 1174 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 7,67 (s, 1H, –CH═), 7.06 (d, J = 3.2 Hz, 1H, C_4H_2O), 6.40 (d, J = 2.2 Hz, 1H, C_4H_2O), 4.97 (q, J = 7.2 Hz, 1H, CH_3CHCO_2H), 2.38 (s, 3H, $CH_3C_4H_2O$), 1.49 (d, J = 3.4 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.5 (CO_2H), 167.9 (C═O), 165.1 (C═O), 158.3 (C_4H_2O), 148.2 (C_4H_2O), 121.7 (–CH═), 120.3 (═C–S), 115.6 (C_4H_2O), 110.8 (C_4H_2O), 50.4 (CH_3CHCO_2H), 14.3 (CH_3CHCO_2H), 14.0 ($CH_3C_4H_2O$) ppm.

3.5.6. 2-(5-(1-Naphthylidene)-2,4-dioxotetrahydrothiazole-3-yl)propionic acid (**12**)

Yellow solid; yield: 48%; m.p.: 163–165 °C; *Anal*. Calcd. for $C_{17}H_{15}NO_4S$ (%): C, 61.99; H, 4.59; N, 4.25. Found (%): C, 70.04; H, 4.62; N, 4.22; FT-IR: ν (KBr): 3446, 2930, 1737, 1663, 1595, 1370, 1227, 1176 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.57 (s, 1H, –CH═), 8.13–8.00 (m, 4H, $C_{10}H_9$), 7.71–7.60 (m, 5H, $C_{10}H_9$), 5.07 (q, J = 7.2 Hz, 1H, CH_3CHCO_2H), 1.55 (d, J = 7.2 Hz, 3H, CH_3CHCO_2H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ = 170.4 (CO_2H), 167.3 (C═O), 164.7 (C═O), 133.5 (–CH═), 131.4 ($C_{10}H_9$), 131.2 ($C_{10}H_9$), 131.0 ($C_{10}H_9$), 130.3 ($C_{10}H_9$), 129.2 ($C_{10}H_9$), 127.8 ($C_{10}H_9$), 127.2 ($C_{10}H_9$), 126.8 ($C_{10}H_9$), 125.9 ($C_{10}H_9$), 124.5 ($C_{10}H_9$), 123.7 (═C–S), 50.6 (CH_3CHCO_2H), 14.2 (CH_3CHCO_2H) ppm.

3.6. In vitro studies

3.6.1. Drugs and solutions

The MTT was dissolved (5 mg/mL) in phosphate buffer saline (pH 7.2) and filtered (0.22 μm) before use. The RPMI 1640 cell culture medium, fetal bovine serum (FBS), and MTT, were purchased from Sigma Chemical Company, USA.

3.6.2. Cell lines

Human colon cancer HCT116 and human breast cancer MDA231 cell lines were maintained in a monolayer culture, and myelogenous leukemia K562 cells in a suspension culture, using a nutrient medium RPMI 1640, with 10% FBS and antibiotics.

3.6.3. Treatment of peritoneal macrophages for evaluation of cytotoxic effect

Stock solutions of compounds were made in dimethyl sulfoxide (DMSO), and were dissolved in corresponding medium to the required working concentrations. The compounds were evaluated for their cytotoxic effects to rat peritoneal macrophages by MTT assay during 24 h. Rat peritoneal macrophages (10 000 cells per well) were seeded into wells of a 96-well flat-bottomed microtiter plate in 100 μL of medium with or without lipopolysaccharide (LPS). After 24 h of incubation, 100 μL of the investigated compound was added to cells in final concentrations (0.01, 0.1, 1, 10, 50, and 100 μM), except in the control wells, where only medium was added to the cells. The effects of compound actions to survival of peritoneal macrophages was determined 24 h later by MTT test (Mosmann, 1983), modified by Ohno and Abe (1991)). Briefly, 20 μL of MTT (5 mg/mL) dye was added to each well. After incubation for further 3 h, 100 μL of 10% SDS (sodium dodecyl sulfate) was added to extract the insoluble product formazan resulting from conversion of the MTT dye by viable cells. The number of viable cells in each well is proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm.

3.6.4. Treatment of cell lines for antiproliferative in vitro screening

Target cells HCT116, MDA231, or K562 cells (in all cases 3000 cells per well) were seeded into wells of a 96-well flat-bottomed microtiter plate in 100 μL medium. Twenty-four hours later, after the cell adaptation for all cell lines and adherence for HCT116 and MDA231 cells, 100 μL of the investigated compound was added to cells in final concentrations (0.01, 0.1, 1, 10, 50, and 100 μM), except in the control wells, where only medium was added to the cells. The effect of substance action on cancer cell survival was determined 24 h later by MTT test. The antiproliferative effect of these drugs was expressed as a percentage of inhibition of untreated cell proliferation. It was calculated as 100 percentages minus ratio between the absorbance of each dose of the compounds and the absorbance of the untreated control cells × 100.

3.6.5. In vitro antimicrobial screening

Overnight cultures of standard strains of microorganisms were used for the preparation of suspensions. Antimicrobial assay was realized by the well-diffusion method (Perez et al., 1990) and the microdilution method with resazurin (Sarker et al., 2007). The diffusion method is a qualitative test which allows the classification of microorganisms as susceptible or resistant to the test substance according to the diameter of the zone of inhibition. The antimicrobial activity was

evaluated by measuring the diameters of the zones of inhibition.

4. Conclusion

In summary, six novel 2-(5-arylidene-2,4-dioxotetrahydrothiazole-3-yl)propanoic acids and six corresponding methyl esters were synthesized and characterized. Almost all of the compounds exhibited significant antiproliferative activity (without non-specific cytotoxic effects) against human colon cancer HCT-116, human breast cancer MDA-231 and human myelogenous leukemia K562 cell lines, especially against HCT-116 cell line. Moreover, all results for antiproliferative activity indicate better biological properties of synthesized esters (**1**–**6**) than the corresponding acids (**7**–**12**). Contrary to the satisfied antiproliferative activities of these compounds, their antimicrobial activity to a wide range of tested microorganisms was not exhibited. These observations might promote a further development and investigation of antiproliferative mechanisms of action of these compounds and might lead to synthesis of compound with anticancer activity as a new potential pharmaceutical agent.

Crystallographic data for the structure **2** were deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 917618. These data can be obtained free of charge from http://www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44–1223-336033.

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