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Synthesis and Antiproliferative Activity in vitro of New Tricyclic 2-Thioxo-1 H ,3 H -imidazo[4,5-b]pyridine Derivatives

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Synthesis and Antiproliferative Activity in vitro of New Tricyclic 2-Thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine Derivatives

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*A series of 2-arylidenethiazolo[2′,3′:2,3]imidazo[4,5-b]pyridine-2-yl-3-ones were prepared by a reaction of 6-bromo- (**4**) or 3H-imidazo[4,5-b]pyridin-2-ylthioglycolic acid (**3**) with appropriate aromatic aldehydes. All new compounds were examined for their antiproliferative activity in vitro against cells of human cancer cell lines, using SRB technique. Preliminary screening data indicated that three of the tested compounds revealed cytotoxic activity.*

Keywords 3*H*-Imidazo[4,5-*b*]pyridin-2-yl-thioglycolic acid; 6-bromo-3*H*-imidazo[4,5-*b*]-pyridin-2-yl-thioglycolic acid; 2-(arylidene)-thiazolo[2′,3′:2,3]imidazo[4,5-*b*]-pyridin-3-ones; antiproliferative activity in vitro

Both thiazole and imidazo[4,5-*b*]pyridine derivatives have been widely claimed to have diverse biological properties, some of which are interesting for the potential treatment of human diseases. Their bioactivity depends on substituents of the heterocyclic ring system and includes antiviral,[1] antibacterial,[2,3] antiulcer,[4,5] antituberculytic,[6] hypoglycemic,[7] anticancer[2,8,9] and anxiolitic[10] action. Compounds with a thiazole, imidazothiazole, or imidazopyridine moiety are capable of generating various reactive intermediates that result in DNA cleavage. DNA degradation plays an important role in chemotherapy and carcinogenesis.[9,11,12]

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Continuing our studies of the chemical and biological properties of imidazo[4,5-*b*]pyridine derivatives[13] and inspired by the effect of fusing the thiazole ring to an aromatic skeleton on the antitumor activity,[9] we synthesized a series of novel tricyclic thiazoloimidazopyridines.

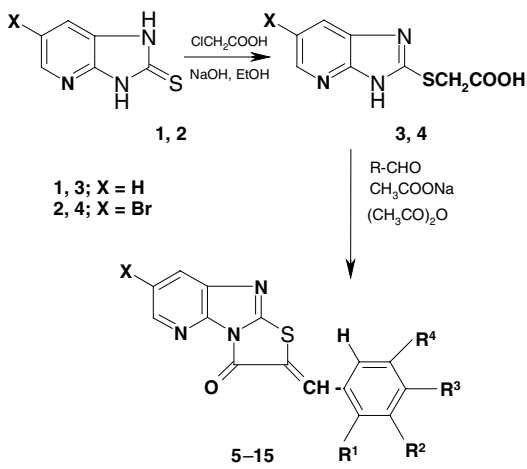
Because a change of the biological activity may be a consequence of even small structural manipulations, thus to broaden the scope of potential cytotoxic activity, a side chain containing substituted arylidene structure was introduced to the triheterocyclic framework. On the basis of some recent reports, because fluorinated analogues improved the biological profile,[14] including cytotoxicity,[15] it seemed interesting to incorporate a fluorine atom into the arylidene structure.

RESULTS AND DISCUSSION

Chemistry

The formation of the fused-ring framework usually takes two or more steps to complete. Substituted imidazopyridines are versatile precursors of fused nitrogen heterocycles.[6,10] The treatment of 2-thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**1**) or 6-bromo-2-thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**2**) with chloroacetic acid in ethanol and the presence of NaOH gave 3*H*-imidazo[4,5-*b*]pyridin-2-yl-thioglycolic acid (**3**) and 6-bromo-3*H*-imidazo[4,5-*b*]pyridin-2-yl-thioglycolic acid (**4**), respectively (Scheme 1). These acids were used as starting compounds for constructing the tricyclic skeleton of the thiazoloimidazopyridine ring system. The preparation of the tricyclic framework followed a pathway previously described for benzoimidazothiazolones.[16] It is a straightforward one-pot approach to furnish the nitrogen heterocyclic framework with a suitable substituent.

When acid **3** or **4** was refluxed in acetic acid anhydride with an appropriate aromatic aldehyde and anhydrous sodium acetate, the reaction resulted in a clean formation of thiazolo[2′,3′:2,3]imidazo[4,5-*b*]-pyridin-3-ones **5–15**. The two-step synthetic route for the substituted triheterocyclic ring system is outlined in Scheme 1. Substituents were chosen that are mostly electron-withdrawing groups because they affect the electron-density distribution pattern what can evoke biological properties of the molecule. The bioactivity of a molecule is based mostly on its capability to bind to an active site; thus, the expected enhancement of biological activity of the obtained compounds could be attributed to the influence of the electron-withdrawing effect, which substituents cause on interaction with a biological receptor or enzyme.[17]



Nº	R^1	R^2	R^3	R^4	X
5	H	H	H	H	H
6	H	H	Cl	H	H
7	H	H	Br	H	H
8	H	H	F	H	H
9	H	H	CF_3	H	H
10	H	NO_2	H	H	H
11	H	H	NO_2	H	H
12	H	H	OCH_3	H	H
13	H	H	OCH_3	OCH_3	H
14	H	OCH_3	OCH_3	OCH_3	H
15	H	H	H	H	Br

SCHEME 1

All new synthesized compounds gave satisfactory elemental analyses, and their molecular structure was confirmed by IR and ^{1}H NMR spectroscopy. Examination of the IR bands for compounds **5–15** showed that the characteristic C=O vibration was in the range $\nu = 1740$–1710 cm^{-1}. ^{1}H NMR spectra of compounds **5–15** showed a characteristic singlet in the region $\delta = 8.5$–8.0 ppm for the olefinic proton =C**H**-Ar, the position of which was sensitive to the presence and nature of substituents in the adjacent phenyl ring. Both steric and electronic effects appear to be important.[18] The introduction of a NO_2 group at the *orto* position (compound **10**) led to a significant deshielding ($\Delta\delta = 0.47$ ppm) of the olefinic proton as compared to the unsubstituted derivative **5**. This observation supports the opinion that electron-attracting groups at the *orto* position in an aromatic ring induce a clear deshielding effect on the α-proton in the side chain. This effect is probably caused by the

electron withdrawal of the substituent transmitted to the side chain, mainly through a *p*-orbital interaction between the α- and *ipso*-carbon atoms.[19] A significant deshielding ($\Delta\delta = 0.38$ ppm) was also observed when two methoxy groups were introduced at the *meta* and *para* positions of the aromatic ring (compound **13**). The introduction of a halogen atom or methoxy group at the *para* position of the phenyl ring (compounds **6–8**, **12**) led to an insignificant deshielding of the olefinic proton. In fact, electronegative atoms and groups exert strong field and inductive effects on a nucleus attached to or near them. This deshielding effect decreases as the number of bonds between the two centers is increased. However, moieties such as nitro and trifluoromethyl groups can influence the electronic properties and thus the intermolecular interactions of an entire molecule. This is confirmed in our observation. The introduction of an NO_2 (**11**) or CF_3 (**9**) group at the *para* position of the phenyl ring causes considerable changes in the electron-density distribution of the whole molecule. For these two compounds, changes in shifts of resonance signals were observed not only for the relatively close olefinic proton but also for remote pyridine protons. This can lead to the conclusion that the thiazole ring, in particular, the sulfur atom, participates in the transmission of the substituent effects from the phenyl ring through the side chain to the imidazopyridine ring system.

Pharmacology

Antiproliferative Activity In Vitro

The compounds **5–15** were examined for their antiproliferative activity in vitro. The following human cancer lines were used: A549 (non-small cell lung cancer) and SW 707 (rectal adenocarcinoma) (Table I). Only compounds **5**, **10**, and **14** revealed weak antiproliferative activity against the cancer cell lines used for this evaluation.

EXPERIMENTAL

Chemistry

Melting points (uncorrected) were measured with a Boethius melting-point apparatus. Analyses were performed on a Perkin Elmer 2400 analyzer, and satisfactory results within $\pm$ 0.4% calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer. ^{1}H NMR spectra were recorded with a Bruker ARX 300 MHz spectrometer at r.t. using DMSO-$d_6$or $CDCl_3$ as solvents, and chemical shifts refer to the residual solvent signal at

TABLE I Antiproliferative Activity of the Compounds AL 5, 10 and 14 Against the Cells of A549 (Nonsmall Cell Lung Cancer) and SW 707 (Rectal Adenocarcinoma)

	ID_{50} [μg/mL $\pm$ SD]	
Compound	A 549	SW 707
5	60.22 ± 26.14	56.57 ± 35.28
10	32.19 ± 12.33	22.81 ± 13.22
14	$30.17 \pm 9{,}07$	27.15 ± 11.12
Cisplatin	0.51 ± 0.03	3.16 ± 0.48
Control		

δ 2.50 or 7.24 ppm, respectively. The course of the reaction, and purity of products were checked by TLC (Kieselgel G, Merck) using diethyl ether:ethanol = 5:1 as an eluent.

Compounds **1** and **2** were preparated as described.[13]

Synthesis of 3H-imidazo[4,5-b]pyridin-2-yl-thioglycolic Acid (3, 4)

0.01 mol 2-thioxo-(**3**) or 5-bromo-2-thioxoimidazo[4,5-*b*]pyridine (**4**) and 1.2 g (0.03 mol) NaOH in 50 mL ethanol was refluxed for 0.5 h. After cooling, 0.01 mol chloroacetic acid was added, and the reaction mixture was refluxed for 2–3 h. The volatile components were removed in vacuo and water (50 mL) was added. The crude product was filtered off, washed with water, and dried.

3H-Imidazo[4,5-b]pyridin-2-yl-thioglycolic Acid (3)

Yield 1.9 g (91%), white solid, m.p. 212–215°C, IR (KBr) ν (cm^{-1}): 3100, 1740, 1580, 1380. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.73 (broad 1H, NH), 8.20 (dd, $J = 4.8$ Hz, 1.2 Hz, 1H, H-5), 7.82 (dd, $J = 7.9$ Hz, 1.2 Hz, 1H, H-7), 7.16 (dd, $J = 7.9$ Hz, 4.8 Hz, 1H, H-6), 4.17 (s, 2H, CH_2). Anal. calcd. for $C_8H_7N_3O_2S$ (209.23): C, 45.92; H, 3.37; N, 20.08%. Found: C, 45.80; H, 3.09; N, 19.94%.

6-Bromo-3H-imidazo[4,5-b]pyridin-2-yl-thioglycolic Acid (4)

Yield: 2.5 g (87%), white solid, m.p. 222–225°C. IR (KBr) ν (cm^{-1}): 3120, 1705, 1580, 1290. ^{1}H NMR (DMSO-d_6) δ (ppm): 11.50 (broad, 1H, NH), 8.26 (d, $J = 2.0$ Hz, 1H, H-5), 8.07 (d, $J = 2.0$ Hz, 1H, H-7), 4.15 (s, 2H, CH_2). Anal. calcd. for $C_8H_6N_3BrO_2S$(288.12): C, 33.35; H, 2.10; N, 14.58%. Found: C, 33.67; H, 1.96; N, 14.34%.

General Procedure for the Synthesis of 2-(Arylidene)-thiazolo[2′,3′:2,3]imidazo[4,5-b]-pyridin-3-ones (5–15)

A mixture of 0.01 mol imidazo[4,5-*b*]pyridin-2-yl-thioglycolic acid (**3** or **4**), 0.01 mol anhydrous sodium acetate, 0.015 mol of the appropriate aromatic aldehyde, and 15 mL acetic acid anhydride was stirred and heated at 120°C (oil bath) for 1.5 h. The precipitate formed was filtered off and crystallized from dimethylformamide.

2-Benzylidenethiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (5)

Yield: 1.4 g (50%), yellow crystals, m.p. 242–245°C. IR (KBr) ν (cm^{-1}): 1730, 1610, 1440, 1400. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.57 (dd, $J = 4.9$ Hz, 1.6 Hz, 1H, H-6), 8.28 (dd, $J = 7.9$ Hz, 1.6 Hz, 1H, H-8), 8.04 (s, 1H, =CH-Ar), 7.54 (m, 5H, Ar-H), 7.29 (dd, $J = 7.9$ Hz, 4.9 Hz, 1H, H-7). Anal. calcd. for $C_{15}H_9N_3OS$ (279.32): C, 64.50; H, 3.25; N, 15.04%. Found: C, 64.19; H, 2.97; N, 14.97%.

2-(4-Chlorobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (6)

Yield: 1.9 g (61%), yellow solid, m.p. 291–293°C. IR (KBr) ν (cm^{-1}): 720, 1605, 1400. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.57 (dd, $J = 5.0$ Hz, 1.6 Hz, 1H, H-6), 8.28 (dd, $J = 7.9$ Hz, 1.6 Hz, 1H, H-8), 8.04 (s, 1H, =CH-Ar), 7.59–7.49 (m, 4H, Ar-H), 7.30 (dd, $J = 7.9$ Hz, 5.0 Hz, 1H, H-7). Anal. calcd. for $C_{15}H_8N_3ClOS$ (313,76): C, 57.42; H, 2.57; N, 13.39%. Found: C, 57.78; H, 2.43; N, 13.61%.

2-(4-Bromobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (7)

Yield: 1.8 g (51%), yellow solid, m.p. 310–312°C. IR (KBr) ν (cm^{-1}): 1720, 1600, 1400. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.57 (dd, $J = 4.0$ Hz, 1.6 Hz, 1H, H-6), 8.28 (dd, $J = 7.9$ Hz, 1.6 Hz, 1H, H-8), 8.03 (s, 1H, =CH-Ar), 7.70–7.66 (m, 2H, Ar-H), 7.50–7.46 (m, 2H, Ar-H), 7.28 (dd, $J = 7.9$ Hz, 4.9 Hz, 1H, H-7). Anal. Calcd. for $C_{15}H_8N_3BrOS$ (358.21): C, 50.29; H, 2.25; N, 11.73%. Found: C, 50.65; H, 2.03; N, 11.76%.

2-(4-Fluorobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (8)

Yield: 1.8 g (60%), yellow solid, m.p. 288–290°C. IR (KBr) ν (cm^{-1}): 1720, 1605, 1400. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.57 (dd, $J = 5.0$ Hz, 1.6 Hz, 1H, H-6), 8.29 (dd, $J = 7.9$ Hz, 1.6 Hz, 1H, H-8), 8.08 (s, 1H, =CH-Ar), 7.65–7.60 (m, 4H, Ar-H), 7.29 (dd, $J = 7.9$ Hz, 5.0 Hz, 1H, H-7). Anal.

calcd. for $C_{15}H_8N_3FOS$ (297.31): C, 60.60; H, 2.71; N, 14.13%. Found: C, 61.02; H, 2.44; N, 14.39%.

2-(4-Trifluorobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (9)

Yield: 1.8 g (52%), yellow solid, m.p. 324–326°C, IR (KBr) ν (cm^{-1}): 1720, 1605, 1400. ^1H NMR ($CDCl_3$) δ (ppm): 8.45 (dd, $J = 4.9$ Hz, 1.3 Hz, 1H, H-6), 8.13 (s, 1H, =CH-Ar), 7.97 (dd, $J = 8.0$ Hz, 1.3 Hz, 1H, H-8), 7.81–7.72 (m, 4H, Ar-H), 7.37 (dd, $J = 8.0$ Hz, 4.9 Hz, 1H, H-7). Anal. calcd. for $C_{16}H_8N_3F_3OS$ (347.31): C, 55.33; H, 2.32; N, 12.10%. Found: C, 55.00; H, 2.19; N, 12.31%.

2-(2-Nitrobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (10)

Yield: 2.1 g (65%), yellow crystals, m.p. 281–283°C. IR (KBr) ν (cm^{-1}): 1730, 1620, 1400. ^1H NMR ($CDCl_3$) δ (ppm): 8.59 (d, $J = 5.0$ Hz, 1H, H-6), 8.51 (s, 1H, =CH-Ar), 8.36 (d, $J = 8.0$ Hz, 1H, H-8), 8.28–8.23 (m, 1H, Ar-H), 7.85–7.64 (m, 3H, Ar-H), 7.36 (dd, $J = 8.0$ Hz, 5.0 Hz 1H, H-7). Anal. calcd. for $C_{15}H_8N_4O_3S$ (324.31): C, 55.55; H, 2.49; N, 17.28%. Found: C, 55.23; H, 2.86; N, 17.01%.

2-(4-Nitrobenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (11)

Yield: 2.3 g (71%), yellow solid, m.p. 347–349°C. IR (KBr) ν (cm^{-1}): 1740, 1605, 1505, 1050. ^1H NMR ($CDCl_3$) δ (ppm): 8.46 (dd, $J = 5.0$ Hz, 1.4 Hz, 1H, H-6), 8.40–8.36 (m, 2H, Ar-H), 8.32–8.29 (m, 2H, Ar-H), 8.17 (s, 1H, =CH), 7.98 (dd, $J = 8.1$ Hz, 1.4 Hz, 1H, H-8), 7.38 (dd, $J = 8.1$ Hz, 5.0, Hz, 1H, H-7). Anal. calcd. for $C_{15}H_8N_4SO_3$ (324.31): C, 55.55, H, 2.49; N, 17.28%. Found: C, 55.86; H, 2.26; N, 17.27%.

2-(4-Methoxybenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (12)

Yield: 2.9 g (74%), yellow solid, m.p. 268–271°C. IR (KBr) ν (cm^{-1}): 1720, 1600, 1440, 1400. ^1H NMR ($CDCl_3$) δ (ppm): 8.55 (d, $J = 4.6$ Hz, 1H, H-6), 8.29 (d, $J = 7.6$ Hz, 1H, H-8), 8.05 (s, 1H, =CH-Ar), 7.58 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.28 (d, $J = 4.6$ Hz, 1H, H-7), 7.03 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.88 (s, 3H, OCH_3) Anal. calcd. for $C_{16}H_{11}N_3O_2S$ (309.34): C, 62.12; H, 3.58; N, 13.58%. Found: C, 62.35; H, 3.37; N, 13.87%.

2-(3,4-Dimethoxybenzylidene)thiazolo[2′,3′,2,3]imidazo[4,5-b]pyridin-3-one (13)

Yield: 2.2 g (65%), yellow solid, m.p. 239–240°C. IR (KBr) ν (cm^{-1}): 1710, 1610, 1400. ^1H NMR ($CDCl_3$) δ (ppm): 8.55 (d, $J = 4.6$ Hz, 1H,

H-6), 8.42 (s, 1H, =CH-Ar), 8.34 (d, $J = 7.6$ Hz, 1H, H-8), 7.51 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.29 (dd, $J = 7.6$ Hz, 4.6 Hz, 1H, H-7), 6.64 (dd, $J = 8.7$ Hz, 2.2 Hz, 1H, Ar-H), 6.49 (d, $J = 2.2$ Hz, 1H, Ar-H), 3.92 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3). Anal. calcd. for $C_{17}H_{13}N_3O_3S$ (339.37): C, 60.17; H, 3.86; N, 12.38%. Found: C, 60.51; H, 3.56; N, 12.40%.

2-(3,4,5-Trimethoxybenzylidene)thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (14)

Yield: 2.4 g (64%), yellow solid, m.p. 207–209°C. IR (KBr) ν (cm^{-1}): 1720, 1600, 1465, 1400. ^1H NMR ($CDCl_3$) δ (ppm): 8.57 (dd, $J = 4.9$ Hz, 1.6 Hz, 1H, H-6), 8.29 (dd, $J = 7.9$ Hz, 4.9 Hz, 1H, H-8), 8.01 (s, 1H, =CH-Ar), 7.28 (dd, $J = 7.9$ Hz, 4.9 Hz, 1H, H-7), 6.98 (s, 2H, Ar-H), 3.94 (s, 6H, $2 \times OCH_3$), 3.92 (s, 3H, OCH_3). Anal. calcd. for $C_{18}H_{15}N_3O_4S$ (369.39): C, 58.53; H, 4.09; N, 11.38%. Found: C, 58.17; H, 3.88; N, 11.57%.

2-Benzylidene-7-bromo-thiazolo[2′,3′:2,3]imidazo[4,5-b]pyridin-3-one (15)

Yield: 2.1 g (58%), yellow crystals, m.p. 329–332°C. IR (KBr) ν (cm^{-1}): 1730, 1600, 1420. ^1H NMR ($CDCl_3$) δ (ppm): 8.48 (s, 1H, H-6), 8.17 (s, 1H, H-8), 8.08 (s, 1H, = CH-Ar), 7.61–7.51 (m, 5H, Ar-H). Anal. calcd. for $C_{15}H_8N_3Br_1O_1S_1$ (358.21): C, 50,29; H, 2.25; N, 11.73%. Found: 50.24; H, 2.08; N, 11.90%.

Biology

Antiproliferative Assay in Vitro

Compounds **5–15** were examined in in vitro screening assay. Test solutions of the compounds (1 mg/mL) were prepared by dissolving the substance in 100 μL DMSO completed with 900 μL tissue culture medium. Afterward, the compounds were diluted in the culture medium to reach the final concentrations of 100, 10, 1, and 0.1 μg/mL.

Cell Lines

The following established in vitro human A549 (nonsmall cell lung carcinoma) and SW707 (rectal adenocarcinoma) cancer cell lines were applied. All cancer cell lines were maintained in culture or frozen in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wrocaw, Poland.

Twentyfour hours before the addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well in 100 μL culture medium. The cells were cultured in the opti-MEM+RPMI-1640 (1:1) medium supplemented with 2 mM gluthamine (Gibco, Warsaw, Poland), streptomycin (50 μg/mL), penicillin (50 U/mL)

(both antibiotics from Polfa, Tarchomin, Poland), and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37°C in a humid atmosphere saturated with 5% CO_2. The results of cytotoxic activity in vitro were expressed as an ID_{50}, which is, the dosis of compound (in μg/mL) that inhibits the proliferation rate of the tumor cells by 50% as compared to the controlled untreated cells.

SRB (Sulphorodamine B)

The details of this technique were described by Skehan et al.[20] The cytotoxicity assay was performed after 72 hours of exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/mL) of the tested agents. Cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4x) with 1% acetic acid. The protein-bound dye was extracted with 10 mM Tris base (POCh, Gliwice, Poland) for a determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter-plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland).

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