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Synthesis, antimicrobial activity and bleaching effect of some reaction products of 4-oxo-4*H*-benzopyran-3-carboxaldehydes with aminobenzothiazoles and hydrazides

Hafez M. El-Shaaer [a], Pavlína Foltínová [b], Margita Lácová [c,*], Jarmila Chovancová [c], Henrieta Stankovičová [c]

[a] *Department of Chemistry, Faculty of Education, Ain Shams University, Cairo, Egypt*
[b] *Institute of Cellular Biology, Comenius University, 81107 Bratislava, Slovak Republic*
[c] *Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University, 84215 Bratislava, Slovak Republic*

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Abstract

The synthesis of the biologically active novel systems derived from reaction of 3-formylchromones with three types of amino derivatives, 6-R^2-2-aminobenzothiazoles, 6-amino-2-R^3-thiobenzothiazoles and hydrazide derivatives (derived from cyanoacetic, isonicotine, salicylic and gallic acids) was carried out. The structures of the prepared compounds have been proved by elemental analysis, ^{1}H NMR and IR spectra. Antimicrobial activity was studied against the following microorganisms — bacteria G$^+$ (*Staphylococcus aureus* 29/58, *Bacillus subtilis* 18/66). G$^-$ (*Escherichia coli* 326/71, *Pseudomonas aeruginosa*); yeasts: *Candida albicans*, *Saccharomyces cerevisiae*; moulds: *Microsporum gypseum*, *Aspergillus niger*, *Scopulariopsis brevicaulis*; and against typical and atypical mycobacteria: *Mycobacterium tuberculosis* ($H_{37}R_v$), *Mycobacterium kansasii* (PFG 8), *Mycobacterium avium* (My 80/72), *Mycobacterium fortuitum* (1021). The hereditary bleaching effect on the plastid system of *Euglena gracilis*, a unique phenomenon of the biological activity of chromone derivatives, is reported. The bleaching test on *E. gracilis* is used for detecting extranuclear mutations.

Keywords: 3-Formylchromone; Bacteria G$^+$ and G$^-$; *Euglena gracilis*; Mycobacteria; Antimicrobial activity

1. Introduction

This paper is a continuation of previous works [1,2] in which synthesis, antimycobacterial activity and photosynthesis inhibition effects of some amino, enamino and imino derivatives of chromones were reported.

The present work describes the preparation of novel systems produced by reactions of 3-formylchromones **1** with 2-aminobenzothiazoles **2**, 6-amino-2-R^3-thiobenzothiazoles **6** and hydrazides **9**, respectively, under different reaction conditions.

The following compounds were prepared: 3-(2-benzothiazolylaminomethylene)-2-ethoxy- **3**, 2-(2-benzothiazolylamino)-3-(2-benzothiazolylaminomethylene)- **5**, 2-ethoxy-3-(2-R^3-thio-6-benzothiazolylaminomethylene)-**6**, 2-(2-R^3-thio-6-benzothiazolylamino)-3-(2-R^3-thio-6-benzothiazolylaminomethylene)-4-chromanone **8** and 3-(2-benzothiazolyliminomethyl)-4-chromone derivatives **4**. The reaction of 3-formylchromones with hydrazides was carried out to compare synthetic and biological results with similar ones of aminobenzothiazole derivatives.

The investigation and evaluation of antimicrobial, antimycobacterial and fungicide activity, and induction of chloroplast-free mutants of *Euglena gracilis* — hereditary bleaching effect — is part of this work.

2. Experimental

2.1. Chemistry

Melting points of the synthesized compounds were determined on a Kofler heating block. The IR spectra were measured on a Specord 75 IR (Zeiss, Jena) apparatus in the region $\nu = 400$–4000 cm^{-1} using suspensions in paraffin oil. ^{1}H NMR spectra of **3b,c** and **4c** were measured on the spectrometer BS 487A (Tesla, 80 MHz) and the spectra of other prepared compounds on the spectrometer Gemini 2000 (Varian, 300 MHz). ^{1}H NMR spectra of compounds **3a** and

* Corresponding author.

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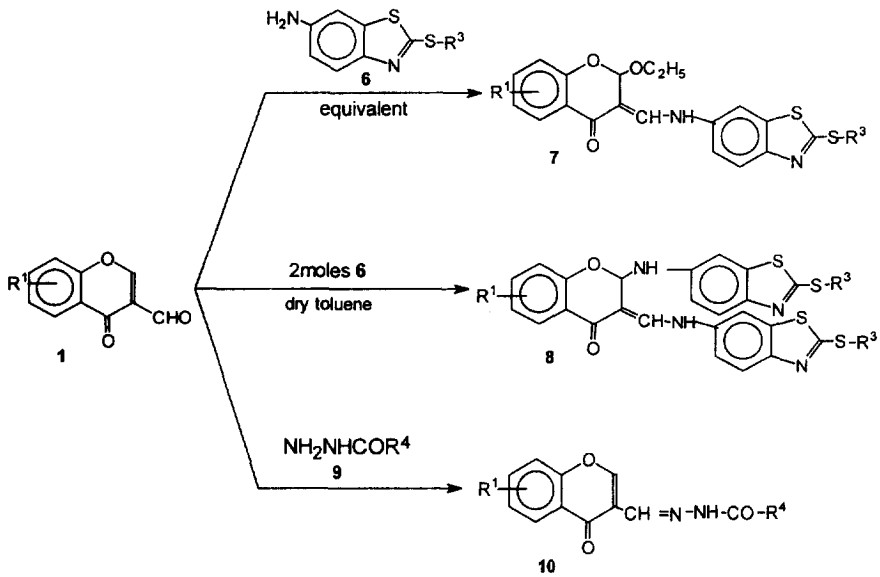
Table 1
Characteristic data of compounds

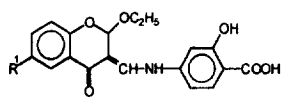
Compound	Formula (mol. wt.)	M.p. (°C)	Analysis: calc. (found)				
			%C	%H	%N	%S	%Cl
3a	$C_{20}H_{18}N_2O_3$ (366.44)	119–121	65.55 (65.57)	4.95 (4.90)	7.64 (7.65)	8.75 (8.73)	
3b	$C_{19}H_{15}ClN_2O_3S$ (386.86)	114–115	58.99 (58.89)	3.91 (3.89)	7.24 (7.28)	8.29 (8.37)	9.16 (9.16)
3c	$C_{19}H_{14}Cl_2N_2O_3S$ (421.30)	253–255	54.17 (54.21)	3.35 (3.32)	6.65 (6.68)	7.61 (5.57)	16.83 (6.62)
3d	$C_{19}H_{14}Cl_2N_2O_3S$ (421.30)	297–299	54.17 (54.17)	3.35 (3.30)	6.65 (6.64)	7.61 (7.51)	16.83 (17.17)
4a	$C_{18}H_{11}ClN_2O_2S$ (354.82)	216–218	60.93 (61.07)	3.12 (3.12)	7.90 (7.83)	9.04 (8.83)	9.99 (10.26)
4b	$C_{17}H_9ClN_2O_2S$ (340.79)	195–197	59.92 (59.74)	2.66 (2.61)	8.22 (8.15)	9.41 (9.51)	10.40 (10.58)
4c	$C_{17}H_8Cl_2N_2O_2S$ (375.23)	246–248	54.42 (54.48)	2.15 (2.12)	7.46 (7.41)	8.54 (8.61)	18.90 (18.87)
4d	$C_{17}H_8Cl_2N_2O_2S$ (375.23)	305–307	54.42 (54.58)	2.15 (2.11)	7.46 (7.44)	8.54 (8.45)	18.90 (19.14)
4e	$C_{17}H_8Cl_2N_2O_2S$ (375.23)	214–216	54.42 (54.16)	2.15 (2.09)	7.46 (7.37)	8.54 (8.38)	18.90 (19.49)
5a	$C_{25}H_{18}N_4O_2S_2$ (470.57)	185–186	63.81 (63.87)	3.85 (3.85)	11.91 (11.68)	13.63 (13.55)	
5b	$C_{24}H_{13}Cl_3N_4O_2S_2$ (559.88)	202–204	51.49 (51.41)	2.34 (2.27)	10.01 (9.86)	11.45 (11.46)	19.00 (19.48)
5c	$C_{24}H_{13}Cl_3N_4O_2S_2$ (559.88)	209–211	51.49 (51.63)	2.34 (2.31)	10.01 (9.81)	11.45 (11.49)	19.00 (18.91)
7a	$C_{19}H_{15}ClN_2O_3S_2$ (418.91)	296–298 decomp.	54.48 (54.39)	3.61 (3.56)	6.69 (6.58)	15.31 (14.81)	8.46 (8.35)
7b	$C_{21}H_{20}N_2O_3S_2$ (412.52)	294–301	61.14 (61.05)	4.89 (4.77)	6.79 (6.65)	15.54 (13.44)	
7c	$C_{25}H_{17}ClN_4O_7S_2$ (585.01)	248–252	51.33 (51.28)	2.93 (2.90)	9.58 (9.45)	10.96 (10.85)	6.06 (5.98)
7d	$C_{26}H_{20}N_4O_7S_2$ (564.59)	222–225	55.31 (55.25)	3.57 (3.50)	9.92 (9.89)	11.36 (11.37)	
7e	$C_{21}H_{20}N_2O_3S_2$ (412.52)	205–207	61.14 (61.10)	4.89 (4.85)	6.79 (6.77)	15.54 (15.71)	
8a	$C_{25}H_{18}N_4O_2S_4$ (534.68)	241–244	56.16 (54.32)	3.39 (3.40)	10.48 (9.89)	23.98 (23.87)	
8b	$C_{26}H_{20}N_4O_2S_4$ (548.71)	199–202	56.91 (56.75)	3.67 (3.65)	10.21 (10.15)	23.37 (23.14)	
10a	$C_{17}H_{12}N_2O_4$ (308.29)	222–224	66.23 (66.35)	3.92 (3.95)	9.09 (8.96)		
10b	$C_{18}H_{14}N_2O_4$ (322.32)	220–222	67.07 (67.28)	4.38 (4.22)	8.69 (8.58)		
10c	$C_{16}H_{11}N_3O_3$ (293.28)	203–206	65.53 (65.64)	3.78 (3.73)	14.33 (14.41)		
10d	$C_{17}H_{13}N_3O_3$ (307.31)	207–209	66.44 (66.69)	4.26 (4.38)	13.67 (13.48)		
10e	$C_{16}H_{10}ClN_3O_3$ (327.73)	208–210	58.64 (58.38)	3.07 (3.16)	12.82 (12.94)		
10f	$C_{17}H_{12}N_2O_6$ (340.29)	255–257	60.00 (60.00)	3.55 (3.54)	8.23 (8.20)		
10g	$C_{17}H_{11}ClN_2O_6$ (374.74)	251–253	54.49 (54.35)	2.96 (2.89)	7.48 (7.45)		
10h	$C_{18}H_{14}N_2O_6$ (354.32)	215–217	61.02 (61.09)	3.98 (4.03)	7.91 (7.74)		
10i	$C_{17}H_{12}N_2O_7$ (356.29)	239–240	57.31 (57.54)	3.39 (3.48)	7.86 (8.14)		
10j	$C_{13}H_9N_3O_5$ (255.23)	226–229	61.18 (61.15)	3.55 (3.48)	16.46 (16.40)		
10k	$C_{13}H_8N_4O_5$ (300.23)	240 decomp.	52.01 (51.80)	2.69 (3.09)	18.66 (18.36)		

4a,**b**,**d**,**e** were not measured due to their very low solubility in DMSO. The characteristic data of the compounds are given in Table 1. 3-Formylchromones **1a–d** were prepared by a published method [3]. Compounds **11** were synthesized for biological tests by a method already described in this journal [1]. The reaction sequences are shown in Schemes 1 and 2.



7	R^1	R^3
a	6-Cl	H
b	6,7-(CH_3)2	H
c	6-Cl	
d	6-CH_3	
e	CH_3	CH_3

8	R^1	R^3
a	6-CH_3	H
b	H	CH_3



11

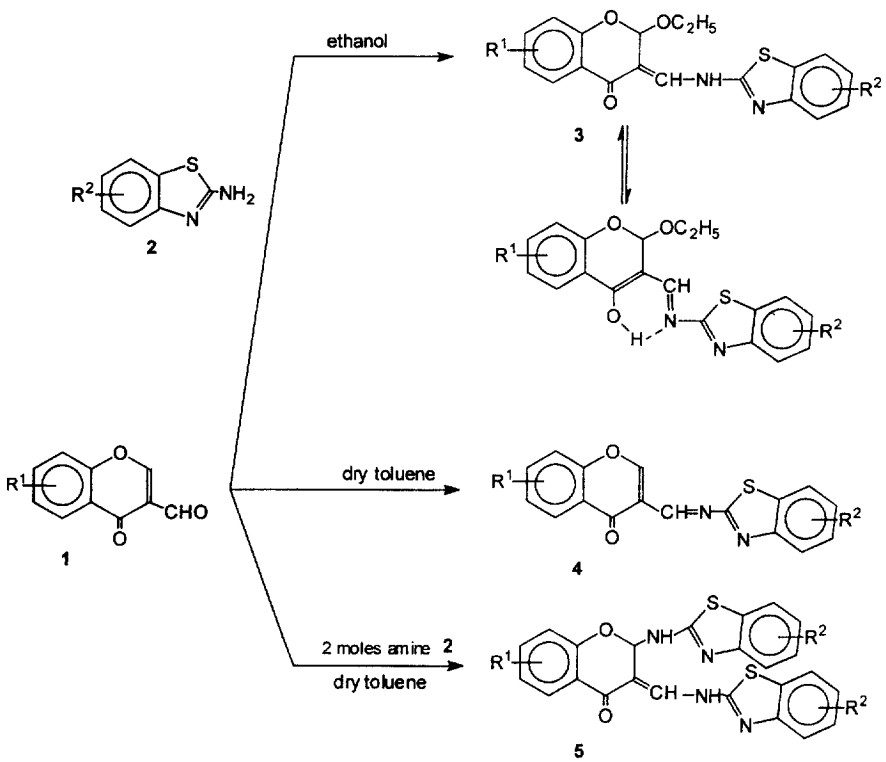
11a	H
11b	Cl

10	R^1	R^4
a	H	
b	6-CH_3	
c	H	
f	H	
g	6-Cl	
h	6-CH_3	
i	6-OH	
j	H	CH_2CN
k	6-NO_2	CH_2CN
d	6-CH_3	
e	6-Cl	

Scheme 1.

*2.1.1. 3-(2-Benzothiazolylaminomethylene)-2-ethoxy-chroman-4-ones **3a–3d** and 2-ethoxy-3-(2-R^3-thio-6-benzo-thiazolylaminomethylene)-chroman-4-ones **7a–7e***

An ethanolic solution of 3-formylchromones **1** (5 mmol) and *p*-toluenesulfonic acid (0.1 mmol) was stirred at 50°C for 30 minutes. After cooling to room temperature, the 2-aminobenzothiazoles **2** (or 6-amino-2-R^3-thiobenzo-thiazoles **6**) in the least amount of absolute ethanol were added to the reaction mixture. The mixture was stirred at room temperature for 2 hours. The solid product was filtered off and recrystallized from absolute ethanol. Yields 70–76%.



3	R^1	R^2
a	6-CH_3	H
b	6-Cl	H
c	6-Cl	4-Cl
d	6-Cl	6-Cl

4	R^1	R^2
a	6-CH_3	4-Cl
b	6-Cl	H
c	6-Cl	4-Cl
d	6-Cl	6-Cl
e	6,8-Cl_2	H

5	R^1	R^2
a	6-CH_3	H
b	6-Cl	4-Cl
c	6-Cl	6-Cl

Scheme 2.

2.1.2. 3-(2-Benzothiazolyliminomethyl)chromones ***4a–e***

A mixture of a solution of 3-formylchromones **1** (5 mmol) in 50 ml of dry toluene, a solution of 2-aminobenzothiazoles **2** (5 mmol) in 80 ml of dry toluene and one crystal of *p*-toluenesulfonic acid was refluxed for 1 h. After concentration of the solvent to half its volume by distillation, the solid was filtered off, boiled in dry toluene, and again filtered off while hot, dried and crystallized from DMSO/ethanol mixture. Yields 50–55%.

2.1.3. 2-(2-Benzothiazolylamino)-3-(2-benzothiazolylamino-methylene)-chroman-4-ones ***5a–c*** *and 2-(2-R^3-thio-6-benzothiazolylamino)-3-(-R^3-thio-6-benzothiazolylamino-methylene)-chroman-4-ones* ***8a,b***

A mixture of 3-formylchromones **1** (5 mmol), 2-amino-benzothiazoles **2** or 6-amino-2-R^3-thiobenzothiazoles **6** (10 mmol), and one crystal of *p*-toluenesulfonic acid in 120 ml dry toluene was refluxed for 1 h. The reaction mixture was concentrated to half its volume by distillation, filtered off, and the solid boiled in dry toluene and filtered off while hot. Products were sufficiently clean for using. They may be crystallized from DMSO. Yields 75–80%.

2.1.4. 3-Formylchromone N-acylhydrazones ***10a–k***

A mixture of a solution of 3-formylchromones **1** (10 mmol) in the least amount of ethanol, a solution of hydrazide derivatives **9** (10 mmol) in ethanol and one crystal of *p*-toluenesulfonic acid was stirred at a temperature of 60°C for 1 h, filtered off while hot and the solid recrystallized from ethanol. Yields 80–82%.

2.2. Microbiology

2.2.1. Antimicrobial activity

All compounds reported herein were tested in vitro for their antimicrobial activity against Gram-positive (*Staphylococcus aureus* 29/58, *Bacillus subtilis* 18/66) and Gram-negative (*Escherichia coli* 326/71, *Pseudomonas aeruginosa*) bacteria as well as yeasts (*Candida albicans* Pn-10, *Saccharomyces cerevisiae*) and moulds (*Microsporum gypseum*, *Aspergillus niger*, *Scopulariopsis brevicaulis*). Antimicrobial activity was tested by the standard plate diffusion method using Mueller-Hinton and Sabouraud agar, or by the standard dilution method in Sabouraud medium [4]. Minimum inhibitory concentrations (MIC) are given in Table 3.

2.2.2. Toxicity and antiplastid activity on Euglena gracilis

The prepared compounds were tested on the autotrophic form of unicellular flagellate *Euglena gracilis* on growth and on the plastid system. This effect was monitored in a Cramer-Myers medium containing appropriate concentrations (10–400 μg/ml) of the tested substances. Inoculum (10^4 cells/ml) was used in its exponential phase of growth. After 4 days of treatment of *E. gracilis* with the compounds, the following data were ascertained: toxicity (ED_{100} and ED_{50} values) and bleaching activity (induction of the chloroplast-free mutants). The method has been described in detail previously [5,6].

2.2.3. Antimycobacterial activity

The experimental method for testing on typical and atypical mycobacteria was used according to Ref. [7]. The strains used here were *Mycobacterium tuberculosis* $H_{37}R_V$ (from CNCTC, Prague) and atypical mycobacteria *Mycobacterium kansasii* PKG 8 (from Dr Runyon, Salt Lake City), *Mycobacterium avium* My 80/72 (from CNCTC Prague) and *Mycobacterium fortuitum* 1021 (from Professor Hauduroy, Lausanne). The tested compounds were dissolved in DMSO at concentrations of 1, 10, 25, 50 and 100 μg/ml. Isoniazid (INH) was used as a reference sample.

2.2.4. Fungicidal activity

The fungicidal tests of chromone derivatives **1b,c**, **3b**, **4b** and **5c** were done according to Ref. [8] by an in vitro method using the following model cultures: *Phytophtora infestans* (on tomatoes), *Alternaria alternata* (agar), *Botrytis cinerea* (agar) and *Fusarium nivale* (agar) at concentration of 100 ppm. Dithane M45, Euparen 50 and Fundazol 50WP served as reference samples.

3. Results and discussion

3.1. Chemistry

Chromones are usually readily ring-opened via nucleophilic attack at the 2-position [9,10]. The presence of a 3-(aryliminomethyl) group alters the reactivity of the system towards nucleophiles and in certain cases facilitates nucleophilic ring addition rather than ring fission [11]. Nucleophiles could be able to react at three electrophilic sites of 3-formylchromones. According to calculated results [12] and spectral study [13,14] the C-2 atom of the 3-formylchromones is the most strongly activated towards nucleophilic reaction. Reaction between equimolar quantities of 3-formylchromone and an aromatic primary amine leads to a mixture of the anil and the 1,4-adduct and making the isolation of the pure compounds difficult [15,16].

The reaction between equimolar quantities of 3-formylchromones **1** and 2-aminobenzothiazoles **2** in dry toluene gave a mixture of anil **4** and the 1,4-adduct **5**. The isolation of the pure anils **4** proved to be difficult, but some of them were obtained by condensation of the 3-formylchromones **1** with the amines **2** in the presence of *p*-toluenesulfonic acid as catalyst.

Excess of the amine **2** or **6** (2:1 molar ratio) produced only the 1,4-adducts **5** and **8**. The reaction between 3-formylchromones and amines **2** and **6** (1:1 molar ratio) in absolute ethanol gave the 1,4-adducts **3** and **7**. The reason for this rather unusual ring-addition of the anils **4** to give adducts **5** and **3** was the formation of the stable oxo-amine hydrogen bond [17]. Ethanol is sufficiently nucleophilic to add to the anils **4** but not sufficiently basic to depronotate the adducts and initiate the elimination. The presence of a small quantity of *p*-toluenesulfonic acid as catalyst is necessary for preparation of all the products in high yields.

Hydrazides **9** produced only condensation products — 3-formylchromone-*N*-acylhydrazones **10**; no adducts with nucleophiles at position 2 of the chromone ring were isolated.

The structure of adducts **3**, **5**, **7** and **8** was confirmed by IR spectra, which indicated a strong band of stretching frequency of the carbonyl group of chroman-4-ones at 1638–1645 cm^{-1}; no evidence was revealed for tautomers with a hydroxy group at position 4. ^1H NMR spectra of the adducts showed a singlet signal of H-2 of the pyrane ring at δ 5.83–6.18 and a doublet signal of H-9 at δ 8.37–8.62. The low δ value of H-2 supports the presence of an H-2 atom on pyranone. Other signals for the ethyl group and Ar–H are given in Table 2.

The compounds **10** were confirmed by IR and ^{1}HNMR spectra. The IR spectra of the hydrazones **10** revealed two separated strong bands resulting from the stretching frequency of CO of the pyrone at 1617–1620 cm^{-1} and from the frequency of the CO amide at 1641–1653 cm^{-1}. Broad bands of NH and OH stretchings, respectively, are at 3153–3251 cm^{-1}. A band for the CN group was observed at 2200 cm^{-1}. A strong band for the carbonyl group of γ-pyrone at 1625 cm^{-1} and a band for the CO amide at 1671–1700 cm^{-1} were found for N-(4-pyridinylcarbonyl)hydrazones **10c–e**. ^1H NMR spectra (in DMSO) of compounds **10** showed a singlet signal of the C=N–NH group at δ 11.65–12.37. ^1H NMR spectra are given in Table 2.

Table 2
^{1}H NMR and IR spectral data of compounds **3–10**

Compound	^{1}H NMR (DMSO): δ (ppm)	IR (cm^{-1})			
		ν(CO)	ν(C=C) or ν(C=N)	$\nu(NH)_{br}$	
3b	11.53 (1H, brs, NH); 8.62–6.75 (8H, m, Ar–H and H-9); 6.14 (1H, s, H-2); 3.82 (2H, q, CH_2); 1.12 (3H, t, CH_3)	1640	1604	3100	
3c	12.37 (1H, d, J = 12.8 Hz, NH); 8.62 (1H, d, J = 12.8 Hz, H-9); 8.31 (1H, d, J = 2.0 Hz, H-5); 7.90–6.90 (5H, m, Ar–H); 5.83 (1H, s, H-2); 3.82 (2H, q, CH_2); 1.20 (3H, t, CH_3)	1640	1600	3112	
3d	11.44 (1H, d, J = 12.0 Hz, NH); 8.37 (1H, d, J = 12.0 Hz, H-9); 8.11 (1H, d, J = 2.2 Hz, H-5); 7.80–7.60 (3H, m, Ar–H); 7.45 (1H, dd, J = 8.7, 2.2 Hz, H-7); 7.18 (1H, d, J = 8.7 Hz, H-8); 6.18 (1H, s, H-2); 3.78 (2H, q, CH_2); 1.07 (3H, t, CH_3)	1639	1600	3100	
4c	8.86 (1H, s, H-2); 8.51–7.29 (7H, m, Ar–H and H-9)	1663	1607		
7a	13.71 (1H, brs, SH); 11.90, 11.86(1H, d, NH); 8.08–7.125 (7H, m, Ar–H); 5.96 (1H, s, H-2); 3.43 (2H, q, CH_2); 1.08 (3H, t, CH_3)	1642	1594	3104	
7b	10.12 (1H, s, NH); 8.02–6.80 (7H, m, Ar–H, SH); 5.85 (1H, s, H-2); 3.455 (2H, q, CH_2); 2.38 (3H, s, CH_3); 2.21 (3H, s, CH_3); 1.06 (3H, t, CH_3)	1650	1610	3310	
7c	10.12 (1H, d, NH); 9.09–6.18 (7H, m, Ar, SH); 6.14 (1H, s, H-2); 3.43 (2H, q, CH_2); 2.51 (3H, s, CH_3); 1.06 (3H, t, CH_3)	1658	1600	3010	ν(CO–C) 1268 $\nu_{as}(NO_2)$ 1580 $\nu_s(NO_2)$ 1338
7d	11.95, 11.92 (1H, d, NH); 8.95–7.07 (10H, m, Ar–H, CH); 6.20, 5.99 (1H, 2s, H-2); 3.45 (2H, q, CH_2); 1.06 (3H, t, CH_3)	1650	1590 1606	3112	ν(CO–C) 1270 $\nu_s(NO_2)$ 1338 $\nu_{as}(NO_2)$ 1570
7e	11.96, 11.92 (1H, d, NH); 8.13–6.96 (7H, m, Ar–H); 5.90 (1H, s, H-2); 3.68 (2H, q, CH_2); 2.80 (3H, s, SCH_3); 2.31(3H, s, CH_3); 1.08 (3H, t, CH_3)	1652	1590 1610	3160	ν(CO–C) 1280
8a	13.45 (2H, brs, SH); 11.85 (2H, 2d, NH); 11.99–8.15–6.80 (10H, m); 6.10 (1H, s, H-2); 2.27 (3H, s, CH_3)	1640	1610	3160	
8b	12.14–11.96 (2H, 2d, NH); 8.20–6.96 (11H, m, Ar); 6.18 (1H, s, H-2); 2.78, 2.74 (6H, 2s, CH_3)	1660	1585 1608	3298	
		ν(CO) pyrone	ν(CO) amide		
10a	11.90 (1H, s, NH); 8.85 (1H, s, H-2); 8.70 (1H, s, H-9); 7.21–8.12 (8H, m, Ar–H)	1620	1653	3251	
10b	11.96 (1H, s, NH); 8.80 (1H, s, H-2); 8.63 (1H, s, H-9); 6.92–8.06 (7H, m, Ar–H); 2.45 (3H, s, CH_3)	1618	1648	3240	
10c	12.16 (1H, s, NH); 8.88 (1H, s, H-2); 8.66 (1H, s, H-9); 8.81–8.78 (2H, dd-py H-2, py H-6); 8.14, 8,13 (2H, d, py H-3; py H-5); 7.57–7.86 (4H, m, Ar–H)	1650	1708	3100 3190	
10d	12.11 (1H, s, NH); 8.82–8.65 (4H, m, H-2, H-9, H-15); 7.64–7.91 (5H, m, Ar–H)	1634	1671	3184	
10e	12.15 (1H, s, NH); 8.74–8.89 (4H, m, H-2, H-9, H-15); 7.84–8.06 (5H, m, Ar–H)	1644	1687	3279	
10f	11.69 (1H, s, NH); 9.2 (3H, brs, H–O); 8.89 (1H, s, H-2); 8.81 (1H, s, H-9); 6.98 (2H, s, H-2′,6′-gall); 7.55–815 (4H, m, Ar–H, pyron)	1620	1645	3110 3280	$\nu(OH)_b$ 3400 $\nu(OH)_f$ 3550
10g	11.70 (1H, s, NH); 9.18 (3H, brs, H–O); 8.81 (1H, s, H-2); 8.56 (1H, s, H-9); 7.79–8.06 (3H, m, Ar); 6.94 (2H, s, H-2′,6′-gall)	1630	1645	3090 3280	$\nu(OH)_b$ 3360 $\nu(OH)_f$ 3510
10h	11.65, 11.31 (1H, 2s, NH); 9.19 (3H, brs, H–O); 8.85 (1H, s, H-2); 8.76 (1H, s, H-9); 7.62–8.52 (3H, m, Ar–H); 6.7–6.9 (2H, m, 2′,6′-gall)	1622	1660	3080 3300	$\nu(OH)_b$ 3400, 3440 $\nu(OH)_f$ 3570
10i	11.64 (1H, s, NH); 10.15 (1H, s, H–O); 9.17 (3H, brs, OH); 8.72 (1H, s, H-2); 8.56 (1H, s, H-9); 7.2–7.6 (3H, m, Ar–H); 6.78–6.93 (2H, m, Ar–H, 2′,6′-gall)	1638	1650	3080 3360 br	
10j	11.67 (1H, brs, NH); 8.84 (1H, s, H-2); 8.43 (1H, s, H-9); 8.15–8.34 (4H, m, Ar–H); 3.43 (2H, s, CH_2)	1628	1660	3140	ν(CN) 2260
10k	11.87 (1H, s, NH); 8.90 (1H, s, H-2); 7.58–8.32 (4H, m, Ar–H, H-9); 3.38 (2H, s, CH_2)	1630	1680	3080 3160 3210	$\nu_s(NO_2)$ 1340 $\nu_{as}(NO_2)$ 1596 ν(CN) 2255

3.2. Microbiology

The new synthesized compounds as well as 3-formylchromones **1a–d** as parent compounds and compounds **11** as chromone derivatives of *p*-aminosalicylic acid were subjected to microbiology and bleaching activity tests. Chromone derivatives of *p*-aminosalicylic acid **11a,b** [1] were prepared for detailed microbiology and bleaching hereditary tests. Their antimycobacterial activity was published in this journal [1].

The results (Table 3) showed that the 3-formylchromones **1** and compounds **11** exhibit interesting activity against G^+ bacteria (MIC 10–50 μg/ml), yeasts (MIC 10–50 μg/ml) and moulds (MIC 100–200 μg/ml). The other derivatives were found to be less effective, while against G^- bacteria they were not effective at all.

Under conditions of active reproduction of *E. gracilis*, the tested substances affect both the cell division and the chloroplast system. The ultimate effect of this action depends upon the chemical structure and the amount of the tested substances. These derivatives exhibit a varying degree of growth inhibition, which is naturally associated with their toxicity. The degree of toxicity is expressed by values ED_{100} and ED_{50} (Table 4). These are calculated from the number of cells obtained after 4 days of treatment with the corresponding concentrations. The 3-formylchromones **1a–d** and derivatives of *p*-aminosalicylic acid **11** showed the highest toxicity (ED_{50} 2.3–24.0) for *E. gracilis* among the derivatives of the given group.

Table 3
Antimicrobial activity of the new synthesized compounds

Compound	MIC (μg/ml)			
	Bacteria [a]		Yeasts [a]	Moulds [a]
	G^+	G^-		
1a	10–50	>400	10–50	100–200
1b	10–50	>400	10–50	100–200
1c	10–50	>400	10–50	100
1d	10–50	>400	10–50	100–200
7a	250	>400	50–250	200–400
7b	250	>400	250	400–>400
7c	250–400	>400	50–250	400–>400
7d	250–400	>400	250	400–>400
10a	400	>400	250–400	400–>400
10b	400	>400	250–400	400–>400
10c	250	>400	250–400	400–>400
10f	400	>400	400–>400	>400
10g	250–400	>400	400–>400	>400
10h	250–400	>400	400	>400
10i	250–400	>400	250–400	>400
10k	400	>400	400	>400
11a	10–50	>400	50	200
11b	10–50	>400	50	200

[a] Bacteria G^+: *S. aureus* 29/58, *Bac. subtilis* 18/66; G^-: *E. coli* 326/71, *Ps. aeruginosa*; yeasts: *Candida albicans* Pn-10, *Saccharomyces cerevisiae*; moulds: *Aspergillus niger*, *Microsporum gypseum*.

Table 4
Effect of synthesized compounds on *Euglena gracilis* — toxicity and bleaching test

Compound	Toxicity (μg/ml)		FM (%) [a] (bleaching active conc. (μg))	
	ED_{100}	ED_{50}	Minimum	Maximum
1a	25.0	2.7	21.0 (5)	81.0 (20)
1b	25.0	2.3	5.0 (5)	76.0 (20)
1c	50.0	24.0		25.8 (25)
1d	25.0	5.5	10.0 (5)	68.0 (20)
7a	350.0	176.3	5.0 (100)	24.4 (200)
7b	400.0	245.5	7.5 (200)	18.6 (300)
7c	150.0	21.3	2.2 (10)	55.5 (100)
7d	150.0	40.4	22.0 (50)	52.3 (100)
10a	400.0	283.0	NB [b]	NB
10b	350.0	265.0	NB	NB
10c	250.0	150.0	6.7 (50)	36.5 (200)
10f	400.0	183.0	NB	NB
10g	400.0	210.0	NB	NB
10h	400.0	205.0	NB	NB
10i	400.0	181.0	NB	NB
10k	350.0	163.0	2.8 (200)	11.3 (300)
11a	25.0	12.3	5.6	20.0 (20)
11b	25.0	12.7	7.4 (5)	28.6 (20)
STM [d]				100.0 (100)

[a] Mutation frequencies — percentage of white mutant colonies per plate, calculated as follows: FM = number of white colonies per plate/number of total colonies per plate.
[b] NB = no bleaching — mutated cells were not observed.
[d] STM — streptomycin as positive control.

The tested substances had an affect on the synthesis of chlorophyll (inhibition 0–55%) and some of them even caused permanent changes in the plastid system — hereditary bleaching. Detection of this phenomenon is very simple, as the permanent loss of functional chloroplasts results in a conversion of the original green colonies to apochlorotic white ones. The highest bleaching activity has been shown by the 3-formylchromones **1a–d**. The maximum frequencies of mutation (FM_{max}) induced by sublethal concentrations are 28.8–81.0% (Table 4).

The mechanism of induction of the chloroplast-free mutants in *E. gracilis* — hereditary bleaching — is not completely understood. We assume that a general condition for the response to the bleaching inducing factor is the higher sensitivity of plastid DNA than that of nuclear DNA. While antibiotics (streptomycin, erythromycin, etc.) and other antibacterial drugs [18,19] induce hereditary bleaching of *E. gracilis* only when they have an effect on dividing cells, mutagens and carcinogens are effective when acting on *E. gracilis* in both non-dividing as well as resting conditions [19,20]. The hereditary bleaching tests on *E. gracilis* are used for detecting extranuclear mutations [19,21,22]. In the case of the chromone derivatives the tests showed positive results only when they had an affect on dividing cells.

Fifteen compounds were evaluated for their in vitro antimycobacterial activity. Antimycobacterial tests showed high activity similar to that of INH in some compounds. Com-

Table 5
Results of the antimycobacterial test (MIC in μg/ml)

Compound	*M. tuberc.*	*M. kansasii*	*M. avium*	*M. fortuitum*
3a	>100	>100	>100	>100
3b	50	100	>100	>100
3d	25	>100	>100	>100
4a	25	50	>100	>100
4c	10	100	>100	>100
4d	10	50	>100	>100
7a	2,5	5	5	>10
7b	5	>5	>5	>10
10b	100	>100	>100	>100
10c	10	>100	>100	>100
10d	1	100	>100	>100
10e	1	>100	>100	>100
10h	>100	>100	>100	>100
10j	10	50	50	>100
10k	10	50	50	>100
INH [a]	<1	25	50	100
Kojic acid [a]	100	100	100	100

[a] Standard samples.

Table 6
Antifungal activity test

Compound	*Phytophtora infestans*	*Alternaria alternata*	*Botrytis cinerea*	*Fusarium nivale*
1b	+	+	+	+
1c	+	+	+	+
3b	N	+	+	+
4b	N	+	+	+
5c	N	–	–	A
S_1 [a]	+	+	N	N
S_2 [a]	N	N	+	N
S_3 [a]	N	N	N	+

Active (+), inactive (–), only active at the beginning (A), not tested (N).
[a] Standards: S_1 = Dithane M45; S_2 = Euparen 50; S_3 = Fundazol 50 WP.

pounds **3a** and **10b,h** showed very low activity, while compounds **10d,c** and **7b,a** showed significant antimycobacterial activity on the same level as that of INH. On the other hand, the other compounds showed a relatively mild increase of activity. Detailed results are given in Table 5.

The results of the fungicide test indicated that the compounds **1b,c** are very interesting because they are active against four types of microorganisms. The compound **4b** is active against *Alternaria* species, *Botrytis cinerea* and *Fusarium nivale*, and other compounds are active against one or two types of microorganism (Table 6).

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