

SYNTHESIS AND BIOLOGICAL EVALUATION OF (E)-3-(NITROPHENYL)-1-(PYRAZIN-2-YL)PROP-2-EN-1-ONES

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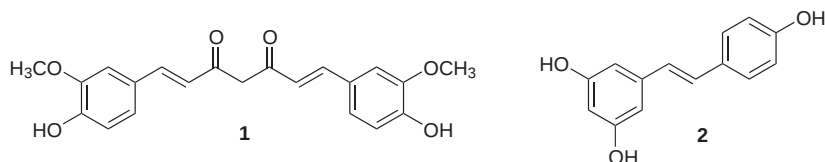
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The title (E)-(3-nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones were prepared by the Claisen-Schmidt condensation of acetylpyrazines and 2-nitro-, 3-nitro- and 4-nitrobenzaldehyde in pyridine using diethylamine as the catalyst. The compounds were bioassayed for in vitro antifungal, antimycobacterial and photosynthesis-inhibiting activity. The high potency of (E)-1-(5-*tert*-butylpyrazin-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one against *Mycobacterium tuberculosis* (MIC 0.78 µg/ml) and moderate activities of several compounds against *Trichophyton mentagrophytes* and *Candida* spp. do not support the assumption that phenolic groups are essential for antimycobacterial and antifungal activity of chalcones and their analogues. In fact, the nitro-substituted compounds were superior to the previously described hydroxylated congeners with antimycobacterial activity (MIC ≥ 12.5 µg/ml). The compounds also reduced chlorophyll content in green alga *Chlorella vulgaris*, and some of them inhibited photosynthetic electron transport in spinach chloroplasts as well. The photosynthesis-inhibiting activity of nitro derivatives was lower than that of the corresponding hydroxylated analogues.

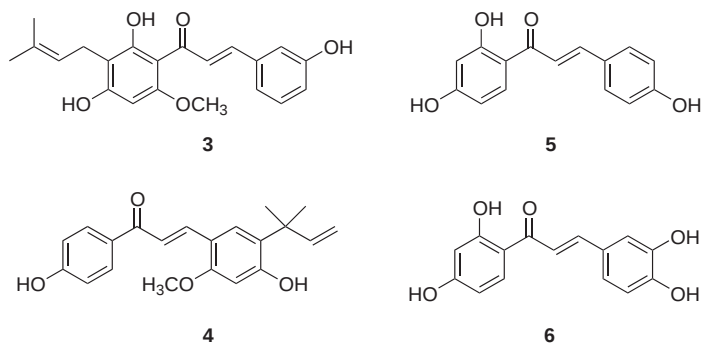
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Chalcones can be regarded as open-chain flavonoids, in which the two aromatic rings are linked by a three-carbon α,β -unsaturated carbonyl system¹. Similar to other compounds with the reactive α,β -unsaturated carbonyl

functionality, they may serve as intermediates in both classical^{2–5} and combinatorial synthesis⁶. From the biological point of view, chalcone (1,3-diphenylprop-2-en-1-one) is a unique template; its derivatives have been the subject of intense research as potential therapeutic agents of serious and often life-threatening diseases. It is worth noting that chalcones are structurally related to curcumin (**1**) and resveratrol (**2**) – highly popular and intensively studied natural products^{7–10}.

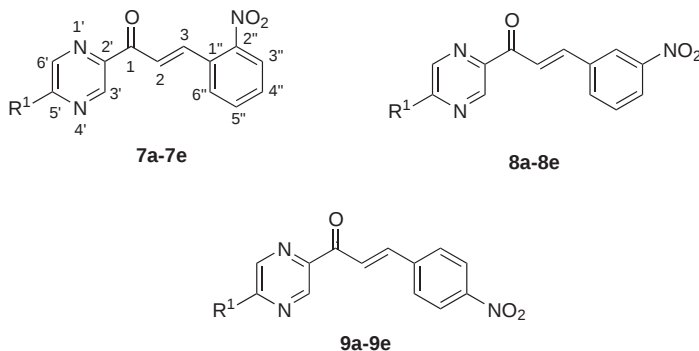


Xanthohumol (**3**), licochalcone A (**4**), isoliquiritigenin (**5**) and butein (**6**) belong to the most intensively studied chalcones. These compounds as well as their synthetic analogues and heterocyclic congeners exhibited antiviral, antibacterial, antiprotozoal, antioxidative, anti-inflammatory, estrogenic, chemoprotective, anticancer and many other interesting biological activities^{11–21}.



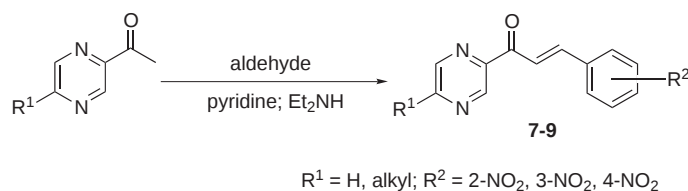
As both tuberculosis and mycoses represent a significant medical problem, search for new antimycobacterial and antifungal compounds is one of the most challenging tasks of current medicinal chemistry^{22–26}. In spite of the progress in understanding the biochemistry of both *Mycobacterium* and various pathogenic fungi as well as the mode of action of current therapeutic agents, it is extremely difficult to find novel compounds that would be superior to established drugs in their activity, and would simultaneously show low toxicity and favourable pharmacological properties. Thus, the structures of the clinically used drugs continue to serve as suitable tem-

plates for the development of new drug candidates. Since pyrazinamide (pyrazine carboxamide) is a first-line drug in tuberculosis therapy²⁴ and antimycobacterial properties of chalcones and their pyridine aza analogues have also been reported¹⁴, we have been interested in isosteric replacement of a phenyl moiety in chalcones with the pyrazine ring. Our previous paper showed that (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones substituted with 3-CH₃O, 4-OH; 2-OH; 4-OH; 4-(CH₃)₂N on the benzene ring possessed both antimycobacterial and antifungal properties²⁷. It was also found that their molecules tend to be planar²⁸, and the enone-moiety plays an important role in their antifungal effects, but does not seem to be necessary for the antimycobacterial activity of these substances²⁹. Based on the well known fact that various nitro-substituted compounds have been widely used as anti-infective and anti-invasive agents, we decided to prepare three new series of (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones bearing a nitro group in position 2 (**7a–7e**), 3 (**8a–8e**) and 4 (**9a–9e**) of the benzene ring. Biological assays showed that antimycobacterial and antifungal potencies of the novel compounds were superior to the previously described series²⁷. Studies concerning the effects of pyrazine derivatives, including (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones, on photosynthetic processes have long-standing tradition among our research efforts, and the results presented in this paper enable us to compare the activities of the nitro-substituted derivatives with those of previously reported compounds^{27,30}.



RESULTS AND DISCUSSION

The title compounds were prepared by the Claisen–Schmidt condensation of acetylpyrazines with nitrobenzaldehydes using a method, reported in our previous paper²⁷. The reaction is illustrated in Scheme 1.



SCHEME 1

Structures of the compounds were corroborated by NMR spectra; all NMR assignments are based on pulse field gradient 2D NMR experiments (gCOSY, gHSQC, and gHMBC). The values of the spin interaction constant J (15–16 Hz) were clearly indicative of *E*-configuration on the double bond. Furthermore, the carbonyl group can assume *s-cis* or *s-trans* conformation with respect to the vinyl double bond^{31,32}. The calculated standard heats of formation for the two conformers of 3-(2-hydroxyphenyl)-1-(pyrazine-2-yl)prop-2-en-1-one indicated that the (*E*)-*s-cis*-conformer was more stable²⁸. Infrared spectra of the propenones **7a–7e**, **8a–8e** and **9a–9e** showed that (*E*)-*s-cis*-conformers ($\nu_{\text{CO}} = 1676\text{--}1665\text{ cm}^{-1}$) with a minor amount of (*E*)-*s-trans*-conformers (a shoulder at $1654\text{--}1635\text{ cm}^{-1}$) were present in the solid state (KBr pellets).

In our previous paper, the importance of phenolic hydroxy groups for antimycobacterial, antifungal and photosynthesis-inhibiting activity was stressed²⁷. The results of this study show that the substitution of the basic skeleton with a phenolic hydroxy group is not necessary for (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones to show antimycobacterial and antifungal activity. In a study performed by Lin and co-workers³³, the highest antimycobacterial activity (MIC = 6.80–31.5 $\mu\text{g/ml}$) was observed with chalcones in which the aryl bound to the carbonyl group was hydroxyphenyl or heteroaryl (pyridin-3-yl, furan-2-yl), and the other ring was lipophilic (phenyl, 3-chlorophenyl, 3-iodophenyl or phenanthren-9-yl). 3-(Nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones reported here also meet these structural criteria and are even more potent. Their MIC values (0.78–6.25 $\mu\text{g/ml}$) are one order of magnitude lower. It is also important to stress that both the compounds prepared by Lin and co-workers and the propenones described in the present work were tested by the Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF). Therefore, the MICs were obtained under the same experimental conditions and are fully comparable. Thus, substitution of the benzene ring with a nitro group seems to be favourable for the antimycobacterial activity.

In order to see whether the difference in lipophilicity could be responsible for the higher antimycobacterial potency of 3-(nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones as compared to 3-(hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones reported in our previous paper²⁷, $\log P$ (the logarithm of the partition coefficient for *n*-octanol/water) and $C \log P$ (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) of both series were calculated by means of ChemDraw Ultra, ver. 7.0 (CambridgeSoft, Cambridge, MA, U.S.A.). It was found that the nitro derivatives are only slightly more lipophilic ($\log P = 1.32\text{--}3.43$; $C \log P = 1.71066\text{--}3.79666$) than the hydroxy derivatives ($\log P = 0.94\text{--}3.07$; $C \log P = 1.30066\text{--}3.38667$). In addition, for the non-alkylated 3-(4-nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-one (**9a**) $\log P = 1.32$ and $C \log P = 1.71066$, for 3-(4-nitrophenyl)-1-(5-*tert*-butylpyrazin-2-yl)prop-2-en-1-one (**9b**) $\log P = 3.43$ and $C \log P = 3.53667$, and for 3-(4-nitrophenyl)-1-(5-propylpyrazin-2-yl)prop-2-en-1-one (**9e**) the calculated lipophilicity parameters are 2.88 and 3.26767, respectively. However, all these compounds exhibited 100% inhibition of *M. tuberculosis* H₃₇Rv in the primary screening test (Table I). In contrast, 2-nitro derivatives **7b–7e** and 3-nitro derivatives **8a–8e** were less effective than the corresponding 4-nitro derivatives **9a–9e** even though the calculated $\log P$ and $C \log P$ values are the same for the three series. Hence, lipophilicity probably plays a minor role in the antimycobacterial properties of chalcones and their heterocyclic analogues, and other structural factors must be taken into account. The nitro group probably gives rise to a favourable electron distribution in the conjugated system of chalcones due to its strong electron-withdrawing properties. This influence is especially obvious with 4-nitro derivatives **9a–9e** that inhibited the growth of *M. tuberculosis* almost completely (85–100%), while the corresponding 4-dimethylamino derivatives²⁷ were practically inactive (0–26%), and 4-hydroxy derivatives²⁷ displayed intermediate potency (35–72%). Regarding substitution of the pyrazine ring, both our previous results²⁷ and high potency of compound **9b** indicate that the presence of a *tert*-butyl group is favourable for antimycobacterial properties of the studied compounds.

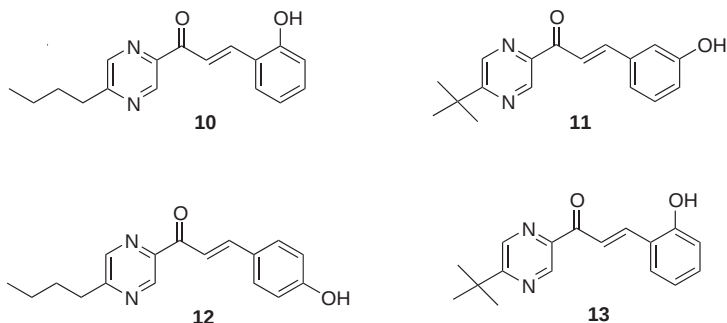
The importance of electron-withdrawing groups in the B-ring of chalcones for their antifungal activity have previously been reported by López and co-workers^{34,35}. For (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones prepared in our laboratory, the dependence of antifungal potency on the substitution was less pronounced. Nevertheless, the nitro derivatives were more potent than their hydroxylated analogues in most cases. Whilst the chalcones studied by López et al. were effective only against dermatophytes^{34,35}, some (*E*)-3-(nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones as

TABLE I
Biological activities of compounds **7a–7e**, **8a–8e** and **9a–9e**

Compd	R ¹	Inhibition of <i>T. mentagrophytes</i> 445 MIC, $\mu\text{mol/l}$		Inhibition of <i>M. tuberculosis</i> H ₃₇ Rv		Inhibition of OER in spinach chloroplasts IC ₅₀ , $\mu\text{mol/l}$	Inhibition of chlorophyll production in <i>C. vulgaris</i> IC ₅₀ , $\mu\text{mol/l}$
		72 h	120 h	% at 6.25 $\mu\text{g/ml}$	MIC, $\mu\text{g/ml}$		
7a	H	31.25	31.25	100	3.13	ND	70.6
7b	<i>tert</i> -butyl	125	500	88	ND	325	ND
7c	isobutyl	15.63	31.25	16	ND	ND	118
7d	butyl	15.63	15.63	43	ND	393	585
7e	propyl	250	250	57	ND	ND	123
8a	H	7.81	15.63	68	ND	658	19.6
8b	<i>tert</i> -butyl	>125	>125	25	ND	461	ND
8c	isobutyl	15.63	15.63	9	ND	340	62.8
8d	butyl	7.81	15.63	17	ND	236	ND
8e	propyl	7.81	15.63	0	ND	ND	18.6
9a	H	15.63	15.63	100	6.25	ND	44.9
9b	<i>tert</i> -butyl	31.25	>62.5	100	0.78	ND	ND
9c	isobutyl	31.25	>62.5	96	>6.25	ND	ND
9d	butyl	15.63	31.25	85	ND	706	ND
9e	propyl	7.81	15.63	100	>6.25	ND	151
10		3.91 ^a	15.63 ^a	33 ^a	ND ^a	184 ^a	ND ^a
11		31.25 ^b	62.5 ^b	28 ^c	ND ^c	105 ^c	238.3 ^c
12		31.25 ^a	62.5 ^a	51 ^a	ND ^a	306 ^a	ND ^a
13		>250 ^a	>250 ^a	94 ^a	12.5 ^a	167 ^a	ND ^a
Ketoconazole		0.24	1.95	ND	ND	ND	ND
Pyrazinamide		ND	ND	0	ND	ND	ND
Rifampicin		ND	ND	98	0.015–0.030	ND	ND
Atrazine		ND	ND	ND	ND	1.00 ^d	0.43 ^e

ND, not determined. ^a Ref.²⁷; ^b ref.³⁶; ^c unpublished results; ^d ref.⁵¹; ^e ref.⁵²

well as their hydroxylated congeners^{27,36}, e.g. **10**, **11** and **12**, were also active against *Candida albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* (MIC = 7.81–125 $\mu\text{mol/l}$). Furthermore, compound **11** inhibited the growth of *Trichosporon beigelii* (MIC = 31.25 $\mu\text{mol/l}$) and *Aspergillus fumigatus* (MIC = 31.25–62.5 $\mu\text{mol/l}$). Among the fungal strains, *Trichophyton mentagrophytes* was the most susceptible to the effects of (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones. Most studied compounds (except for **7b**, **7e** and **8b**) exhibited moderate potency against this strain. In contrast to antimycobacterial activity, substitution of the pyrazine ring with *tert*-butyl group seems to be unfavorable for the inhibitory effect against *T. mentagrophytes* (see the MICs of compounds **7b**, **8b** and the reference compound **13** in Table I).



In the oxygen evolution rate (OER) study with spinach chloroplasts, poor water solubility of the compounds was a serious problem (most compounds precipitated after adding their solutions in DMSO to the suspensions of chloroplasts). Consequently, only a few data could be obtained. Nevertheless, the comparison of IC_{50} values (Table I) of compounds **7b**, **7d** and **9d** with those reported for the previously tested comparable derivatives **13**, **10** and **12**²⁷ clearly shows that nitro derivatives were approximately half as active as the corresponding hydroxylated compounds. The decreased aqueous solubility limiting the passage of the compounds through the hydrophilic regions of thylakoid membranes, and hence a reduced access to their site of action is probably responsible for the observed activity decrease. The studied 3-(2-nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones **7a**, **7c**, **7d**, **7e**, 3-(3-nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones **8a**, **8c**, **8e** as well as the 4-nitro compounds **9a** and **9e** inhibited chlorophyll production in *Chlorella vulgaris* (Table I). Due to low aqueous solubility of the remaining compounds, the corresponding IC_{50} values could not be determined. In the studied concentration range 0.83–100 $\mu\text{mol/l}$, these compounds caused ap-

proximately 7–15% reduction in chlorophyll content in the suspension of *Chlorella vulgaris*.

In spite of much research effort devoted to chalcones and their heterocyclic analogues, little is known about their mechanisms of action at the molecular level. In the photosynthetic apparatus of spinach chloroplasts, these compounds probably interact with the tyrosine radical in position 161 (Tyr_D), located in D₂ protein at the donor site of photosystem 2, which results in the inhibition of the photosynthetic electron transport³⁷. The reduction of chlorophyll content in *Chlorella vulgaris* in the presence of the studied compounds could have been caused by the inhibition of biochemical processes associated with the biosynthesis of this important photosynthetic pigment and/or other constituents of the plant cell, e.g. proteins or lipids. Moreover, the inhibition of the photosynthetic electron transport in *Chlorella vulgaris* by a series of related ring-substituted (*E*)-3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones was reported previously²⁷. Regarding antifungal activities, it was suggested that chalcone-like compounds might act on the fungal cell membranes³⁸. Inhibition of β (1,3)-glucan and chitin synthases by chalcone derivatives has recently been reported³⁴. It is well known that α,β -unsaturated carbonyl compounds can react with nucleophiles, e.g. thiol or amino groups of enzymes, to give Michael-addition type products³⁹. Hence, enzyme inhibition might play a more general role in the biological effects of chalcones and their heterocyclic analogues. To the best of our knowledge, no study aimed at determining the molecular mechanisms of antimycobacterial effects of chalcones and their analogues has been published, even though their antimycobacterial properties have been known since the 1950's⁴⁰. Since then only a few papers concerning the effects of chalcone-like compounds on mycobacteria^{33,41–44} have appeared, and the relationships between their structure and antimycobacterial activity remain to be defined.

The results obtained in this study clearly show that chalcones and their heterocyclic analogues bearing electron-withdrawing groups in the B-ring deserve further attention as potential antituberculous and antimycotic agents. The best antimycobacterial activity was found with (*E*)-1-(5-*tert*-butylpyrazin-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**9b**), and the best antifungal properties were exhibited by (*E*)-3-(3-nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones (**8a**, **8d** and **8e**). Compound **8a** was effective not only against *T. mentagrophytes*, but also against *Candida* spp. (MIC = 7.81–15.63 μ mol/l). Further testing and possible development of these compounds will depend on the results of cytotoxicity assays. In the tests performed in TAACF, evaluation of cytotoxicity has been complicated by the poor solu-

bility in the tissue culture medium. Nevertheless, the calculated lipophilicity parameters indicate that the compounds meet the criteria⁴⁵ set by the Lipinski's rule of 5', and if need arises, the solubility problems could be resolved using modern approaches of pharmaceutical technology.

EXPERIMENTAL

Acetylpyrazines prepared as described previously⁴⁶ and commercially available nitrobenzaldehydes were used as the starting materials. Silpearl (Kavalier, Votice) was used for flash column chromatography. The purity of the products was checked by TLC on Silufol UV 254 plates (Kavalier, Votice). The following solvent mixtures were used for TLC: light petroleum–ethyl acetate 60:40 (v/v) and light petroleum–ethyl acetate 80:20 (v/v). Analytical samples were dried over anhydrous phosphorus pentoxide under reduced pressure at room temperature. Melting points were determined on a Boëtius apparatus and are uncorrected. Elemental analyses were performed on an EA 1110 CHNS instrument (CE Instruments, Milano, Italy). Infrared spectra were recorded in KBr pellets on a Nicolet Impact 400 IR spectrophotometer. Characteristic wavenumbers are given in cm^{-1} . ^1H and ^{13}C NMR spectra were recorded in CDCl_3 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C . Chemical shifts were recorded as δ values in ppm, and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal (7.26 for ^1H , 77.0 for ^{13}C in CDCl_3). Coupling constants J are given in Hz.

Preparation of Compounds **7a–7e**, **8a–8e** and **9a–9e**. General Procedure

Acetylpyrazine (0.01 mol) and a nitrobenzaldehyde (0.01 mol) were dissolved in pyridine (4.4 ml). Diethylamine (0.73 g, 0.01 mol) was added, and the reaction mixture was stirred at 80–120 °C for 2 h. After cooling, the mixture was poured into ice water (200 ml), acidified to pH 3 with a few drops of acetic acid, and then refrigerated for 24 h. The separation of crude products from water depended on their character. Solids were filtered off and crystallized while oily mixtures were extracted with diethyl ether and subjected to flash chromatography on silica gel. Light petroleum–ethyl acetate 60:40 (v/v) was used as the eluent for compounds **7a–7e**, whilst 80:20 (v/v) ratio of the two solvents proved to be more suitable for compounds **8a–8e** and **9a–9e**. The fractions containing the desired compounds were combined and crystallized. Repeated crystallization from absolute ethanol was sufficient to obtain analytically pure crystals of compounds **7a–7e** and **8a–8e**. Compounds **9a–9e** had to be crystallized from acetone to remove the starting 4-nitrobenzaldehyde completely. The final crystallization was then performed from absolute ethanol.

(*E*)-3-(2-Nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-one (**7a**). Yield 22%, m.p. 144–145 °C (ref.³¹ 196–198 °C). For $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_3$ (255.2) calculated: 61.18% C, 3.55% H, 16.46% N; found: 60.93% C, 3.83% H, 16.50% N. IR: 1671 (C=O), 1608 (CH=CH). ^1H NMR: 8.07 d, 1 H, J = 15.9 (H-2); 8.39 d, 1 H, J = 15.9 (H-3); 9.39 d, 1 H, J = 1.5 (H-3'); 8.80 d, 1 H, J = 2.5 (H-5'); 8.69 dd, 1 H, J = 1.5 and 2.5 (H-6'); 8.07 dd, 1 H, J = 1.4 and 7.9 (H-3''); 7.58 td, 1 H, J = 1.4 and 7.9 (H-4''); 7.70 td, 1 H, J = 1.4 and 7.9 (H-5''); 7.86 dd, 1 H, J = 1.4 and 7.9 (H-6''). ^{13}C NMR: 188.7 (C-1); 124.7 (C-2); 140.6 (C-3); 147.8 (C-2'); 144.9 (C-3'); 147.6 (C-5'); 143.3 (C-6'); 130.9 (C-1''); 148.8 (C-2''); 124.9 (C-3''); 130.6 (C-4''); 133.4 (C-5''); 129.2 (C-6'').

(*E*)-1-(5-*tert*-Butylpyrazin-2-yl)-3-(2-nitrophenyl)prop-2-en-1-one (**7b**). Yield 20%, m.p. 131–134 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.16% C, 5.36% H, 13.32% N. IR: 1672 (C=O), 1607 (CH=CH). 1H NMR: 8.04 d, 1 H, $J = 15.9$ (H-2); 8.33 d, 1 H, $J = 15.9$ (H-3); 9.26 d, 1 H, $J = 1.4$ (H-3'); 8.71 d, 1 H, $J = 1.4$ (H-6'); 8.03 dd, 1 H, $J = 1.3$ and 7.9 (H-3''); 7.56 td, 1 H, $J = 1.3$ and 7.9 (H-4''); 7.67 td, 1 H, $J = 1.3$ and 7.9 (H-5''); 7.84 dd, 1 H, $J = 1.3$ and 7.9 (H-6''); 1.43, 9 H (*tert*-butyl). ^{13}C NMR: 188.1 (C-1); 125.1 (C-2); 140.1 (C-3); 145.4 (C-2'); 143.6 (C-3'); 168.2 (C-5'); 140.2 (C-6'); 131.3 (C-1''); 149.0 (C-2''); 125.4 (C-3''); 130.7 (C-4''); 133.6 (C-5''); 129.5 (C-6''); 30.0, 37.5 (*tert*-butyl).

(*E*)-1-(5-Isobutylpyrazin-2-yl)-3-(2-nitrophenyl)prop-2-en-1-one (**7c**). Yield 16%, m.p. 103–104 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.66% C, 5.64% H, 13.75% N. IR: 1673 (C=O), 1608 (CH=CH). 1H NMR: 8.06 d, 1 H, $J = 15.9$ (H-2); 8.36 d, 1 H, $J = 15.9$ (H-3); 9.30 d, 1 H, $J = 1.5$ (H-3'); 8.48 d, 1 H, $J = 1.5$ (H-6'); 8.05 dd, 1 H, $J = 1.4$ and 8.0 (H-3''); 7.57 td, 1 H, $J = 1.4$ and 8.0 (H-4''); 7.68 td, 1 H, $J = 1.4$ and 8.0 (H-5''); 7.85 dd, 1 H, $J = 1.4$ and 8.0 (H-6''); 0.97 d, 6 H, $J = 6.6$, 2.18 m, 1 H, 2.79 d, 2 H, $J = 7.1$ (isobutyl). ^{13}C NMR: 187.9 (C-1); 125.0 (C-2); 140.1 (C-3); 145.5 (C-2'); 144.2 (C-3'); 160.8 (C-5'); 143.2 (C-6'); 131.0 (C-1''); 148.8 (C-2''); 124.9 (C-3''); 130.5 (C-4''); 133.4 (C-5''); 129.3 (C-6''); 22.4, 29.2, 44.8 (isobutyl).

(*E*)-1-(5-Butylpyrazin-2-yl)-3-(2-nitrophenyl)prop-2-en-1-one (**7d**). Yield 20%, m.p. 75–76 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.66% C, 5.60% H, 13.66% N. IR: 1668 (C=O), 1611 (CH=CH). 1H NMR: 8.05 d, 1 H, $J = 15.8$ (H-2); 8.36 d, 1 H, $J = 15.8$ (H-3); 9.29 d, 1 H, $J = 1.5$ (H-3'); 8.52 d, 1 H, $J = 1.5$ (H-6'); 8.06 dd, 1 H, $J = 1.4$ and 8.0 (H-3''); 7.57 td, 1 H, $J = 1.4$ and 8.0 (H-4''); 7.69 td, 1 H, $J = 1.4$ and 8.0 (H-5''); 7.85 dd, 1 H, $J = 1.4$ and 8.0 (H-6''); 0.96, 3 H, $J = 7.3$, 1.42 m, 2 H, 1.78 m, 2 H, 2.93 t, 2 H, $J = 8.0$ (butyl). ^{13}C NMR: 187.8 (C-1); 125.1 (C-2); 140.0 (C-3); 145.5 (C-2'); 144.1 (C-3'); 161.6 (C-5'); 142.7 (C-6'); 131.0 (C-1''); 148.7 (C-2''); 124.9 (C-3''); 130.4 (C-4''); 133.3 (C-5''); 129.2 (C-6''); 13.9, 22.5, 31.4, 35.6 (butyl).

(*E*)-3-(2-Nitrophenyl)-1-(5-propylpyrazin-2-yl)prop-2-en-1-one (**7e**). Yield 21%, m.p. 129–131 °C. For $C_{16}H_{15}N_3O_3$ (297.3) calculated: 64.64% C, 5.09% H, 14.13% N; found: 64.38% C, 5.17% H, 14.26% N. IR: 1671 (C=O), 1606 (CH=CH). 1H NMR: 8.05 d, 1 H, $J = 15.9$ (H-2); 8.34 d, 1 H, $J = 15.9$ (H-3); 9.27 d, 1 H, $J = 1.5$ (H-3'); 8.51 d, 1 H, $J = 1.5$ (H-6'); 8.04 dd, 1 H, $J = 1.3$ and 7.8 (H-3''); 7.56 td, 1 H, $J = 1.3$ and 7.8 (H-4''); 7.68 td, 1 H, $J = 1.3$ and 7.8 (H-5''); 7.84 td, 1 H, $J = 1.3$ and 7.8 (H-6''); 1.00 t, 3 H, $J = 7.3$, 1.82 m, 2 H, 2.89 t, 2 H, $J = 8.0$ (propyl). ^{13}C NMR: 187.8 (C-1); 125.0 (C-2); 140.0 (C-3); 145.5 (C-2'); 144.1 (C-3'); 161.4 (C-5'); 142.8 (C-6'); 131.0 (C-1''); 148.7 (C-2''); 124.8 (C-3''); 130.4 (C-4''); 133.3 (C-5''); 129.2 (C-6''); 13.8, 22.6, 37.8 (propyl).

(*E*)-3-(3-Nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-one (**8a**). Yield 42%, m.p. 165–168 °C (ref.³¹ 166–168 °C). For $C_{13}H_9N_3O_3$ (255.2) calculated: 61.18% C, 3.55% H, 16.46% N; found: 61.13% C, 3.65% H, 16.34% N. IR: 1676 (C=O), 1609 (CH=CH). 1H NMR: 7.97 d, 1 H, $J = 16.1$ (H-2); 8.30 d, 1 H, $J = 16.1$ (H-3); 9.38 d, 1 H, $J = 1.5$ (H-3'); 8.81 d, 1 H, $J = 2.5$ (H-5'); 8.72 dd, 1 H, $J = 1.5$ and 2.5 (H-6'); 8.57 t, 1 H, $J = 1.9$ (H-2''); 8.02–7.96 m, 1 H (H-4''); 7.62, 1 H, $J = 8.0$ (H-5''); 8.29–8.24 m, 1 H (H-6''). ^{13}C NMR: 188.1 (C-1); 125.0 (C-2); 142.3 (C-3); 147.8 (C-2'); 144.9 (C-3'); 147.8 (C-5'); 143.4 (C-6'); 136.1 (C-1''); 122.7 (C-2''); 148.7 (C-3''); 122.8 (C-4''); 134.6 (C-5''); 130.0 (C-6'').

(*E*)-1-(5-*tert*-Butylpyrazin-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (**8b**). Yield 40%, m.p. 160–162 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.21% C, 5.56% H, 13.70% N. IR: 1676 (C=O), 1610 (CH=CH). 1H NMR: 7.95 d, 1 H, $J = 15.9$ (H-2); 8.30 d, 1 H, $J = 15.9$ (H-3); 9.29 d, 1 H, $J = 1.7$ (H-3'); 8.76 d, 1 H, $J = 1.7$ (H-6');

8.57 t, 1 H, $J = 1.8$ (H-2''); 8.02–7.96 m, 1 H (H-4''); 7.62 t, 1 H, $J = 8.0$ (H-5''); 8.29–8.23 m, 1 H (H-6''); 1.46 s, 9 H (*tert*-butyl). ^{13}C NMR: 188.2 (C-1); 124.8 (C-2); 140.0 (C-3); 145.2 (C-2'); 143.4 (C-3'); 168.2 (C-5'); 141.8 (C-6'); 136.6 (C-1''); 122.8 (C-2''); 148.7 (C-3''); 123.2 (C-4''); 134.5 (C-5''); 130.0 (C-6''); 29.7, 37.2 (*tert*-butyl).

(*E*)-1-(5-Isobutylpyrazin-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (**8c**). Yield 40%, m.p. 103–104 °C. For $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.23% C, 5.56% H, 13.65% N. IR: 1676 (C=O), 1610 (CH=CH). ^1H NMR: 7.95 d, 1 H, $J = 15.9$ (H-2); 8.30 d, 1 H, $J = 15.9$ (H-3); 9.30 d, 1 H, $J = 1.5$ (H-3'); 8.52 d, 1 H, $J = 1.5$ (H-6'); 8.58 t, 1 H, $J = 2.2$ (H-2''); 8.02–7.96 m, 1 H (H-4''); 7.62 t, 1 H, $J = 8.0$ (H-5''); 8.29–8.23 m, 1 H (H-6''); 0.98 d, 6 H, $J = 6.9$, 2.27–2.11 m, 1 H, 2.80 d, 2 H, $J = 7.1$ (isobutyl). ^{13}C NMR: 188.2 (C-1); 124.9 (C-2); 141.9 (C-3); 145.6 (C-2'); 144.1 (C-3'); 161.0 (C-5'); 143.4 (C-6'); 136.6 (C-1''); 122.8 (C-2''); 148.7 (C-3''); 123.1 (C-4''); 134.6 (C-5''); 130.0 (C-6''); 22.3, 29.2, 44.8 (isobutyl).

(*E*)-1-(5-Butylpyrazin-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (**8d**). Yield 30%, m.p. 102–104 °C. For $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.15% C, 5.56% H, 13.76% N. IR: 1672 (C=O), 1609 (CH=CH). ^1H NMR: 7.95 d, 1 H, $J = 16.1$ (H-2); 8.30 d, 1 H, $J = 16.1$ (H-3); 9.28 d, 1 H, $J = 1.4$ (H-3'); 8.55 d, 1 H, $J = 1.4$ (H-6'); 8.57 t, 1 H, $J = 2.0$ (H-2''); 8.01–7.96 m, 1 H (H-4''); 7.62 t, 1 H, $J = 8.0$ (H-5''); 8.26 ddd overlapped, 1 H, $J = 8.24$ and 2.0 and 1.1 (H-6''); 0.96 t, 3 H, $J = 7.6$, 1.50–1.35 m, 2 H, 1.84–1.72 m, 2 H, 2.93 t, 2 H, $J = 7.6$ (butyl). ^{13}C NMR: 188.2 (C-1); 124.8 (C-2); 141.9 (C-3); 145.6 (C-2'); 144.1 (C-3'); 161.9 (C-5'); 142.9 (C-6'); 136.6 (C-1''); 122.8 (C-2''); 148.7 (C-3''); 123.1 (C-4''); 134.6 (C-5''); 130.0 (C-6''); 13.8, 22.4, 31.4, 35.6 (butyl).

(*E*)-3-(3-Nitrophenyl)-1-(5-propylpyrazin-2-yl)prop-2-en-1-one (**8e**). Yield 18%, m.p. 91–92 °C. For $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_3$ (297.3) calculated: 64.64% C, 5.09% H, 14.13% N; found: 64.70% C, 5.20% H, 14.13% N. IR: 1669 (C=O), 1609 (CH=CH). ^1H NMR: 7.90 d overlapped, 1 H, $J = 15.8$ (H-2); 8.30 d overlapped, 1 H, $J = 15.8$ (H-3); 9.28 d, 1 H, $J = 1.6$ (H-3'); 8.55 d, 1 H, $J = 1.6$ (H-6'); 8.57 t, 1 H, $J = 2.1$ (H-2''); 8.29–8.22 m, 1 H (H-4''); 7.62 t, 1 H, $J = 8.0$ (H-5''); 7.95–8.00 m, 1 H (H-6''); 1.01 t, 3 H, $J = 7.3$, 1.90–1.75 m, 2 H, 2.91 t, 2 H, $J = 7.6$ (propyl). ^{13}C NMR: 188.2 (C-1); 124.8 (C-2); 141.9 (C-3); 145.6 (C-2'); 144.1 (C-3'); 161.6 (C-5'); 142.9 (C-6'); 136.6 (C-1''); 122.8 (C-2''); 148.7 (C-3''); 123.1 (C-4''); 134.6 (C-5''); 130.0 (C-6''); 13.8, 22.6, 37.7 (propyl).

(*E*)-3-(4-Nitrophenyl)-1-(pyrazin-2-yl)prop-2-en-1-one (**9a**). Yield 43%, m.p. 188–192 °C (ref.³¹ 189–192 °C). For $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_3$ (255.2) calculated: 61.18% C, 3.55% H, 16.46% N; found: 61.47% C, 3.67% H, 16.22% N. IR: 1672 (C=O), 1612 (CH=CH). ^1H NMR: 7.96 d, 1 H, $J = 16.2$ (H-2); 8.30 d, 1 H, $J = 16.2$ (H-3); 9.39 d, 1 H, $J = 1.5$ (H-3'); 8.82 d, 1 H, $J = 2.6$ (H-5'); 8.72 dd, 1 H, $J = 1.5$ and 2.6 (H-6'); 7.86 d, 2 H, $J = 8.6$ (H-2'' and H-6''); 8.28, 2 H, $J = 8.6$ (H-3'' and H-5''). ^{13}C NMR: 188.1 (C-1); 123.8 (C-2); 142.2 (C-3); 147.9 (C-2'); 144.7 (C-3'); 147.8 (C-5'); 143.4 (C-6'); 140.8 (C-1''); 129.4 (C-2'' and C-6''); 124.2 (C-3'' and C-5''); 148.7 (C-4'').

(*E*)-1-(5-*tert*-Butylpyrazin-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**9b**). Yield 43%, m.p. 142–144 °C. For $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.74% C, 5.69% H, 13.75% N. IR: 1672 (C=O), 1613 (CH=CH). ^1H NMR: 7.92 d, 1 H, $J = 16.0$ (H-2); 8.28 d, 1 H, $J = 16.0$ (H-3); 9.27 d, 1 H, $J = 1.5$ (H-3'); 8.73 d, 1 H, $J = 1.5$ (H-6'); 7.84 d, 2 H, $J = 8.8$ (H-2'' and H-6''); 8.26 d, 2 H, $J = 8.8$ (H-3'' and H-5''); 1.44 s, 9 H (*tert*-butyl). ^{13}C NMR: 188.1 (C-1); 124.1 (C-2); 141.6 (C-3); 145.1 (C-2'); 143.4 (C-3'); 168.2 (C-5'); 139.9 (C-6'); 140.9 (C-1''); 129.2 (C-2'' and C-6''); 124.1 (C-3'' and C-5''); 148.6 (C-4''); 29.7, 37.2 (*tert*-butyl).

(*E*)-1-(5-Isobutylpyrazin-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**9c**). Yield 45%, m.p. 141–143 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.23% C, 5.67% H, 13.38% N. IR: 1672 (C=O), 1610 (CH=CH). 1H NMR: 7.94 d, 1 H, J = 16.0 (H-2); 8.30 d, 1 H, J = 16.0 (H-3); 9.29 d, 1 H, J = 1.5 (H-3'); 8.51 d, 1 H, J = 1.5 (H-6'); 7.85 d, 2 H, J = 8.8 (H-2'' and H-6''); 8.27 d, 2 H, J = 8.8 (H-3'' and H-5''); 0.97 d, 6 H, J = 6.6, 2.18 m, 1 H, 2.80 d, 2 H, J = 7.3 (isobutyl). ^{13}C NMR: 188.2 (C-1); 124.2 (C-2); 141.7 (C-3); 145.5 (C-2'); 144.2 (C-3'); 161.1 (C-5'); 143.4 (C-6'); 140.9 (C-1''); 129.3 (C-2'' and C-6''); 124.2 (C-3'' and C-5''); 148.7 (C-4''); 22.4, 29.2, 44.8 (isobutyl).

(*E*)-1-(5-Butylpyrazin-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**9d**). Yield 40%, m.p. 117–119 °C. For $C_{17}H_{17}N_3O_3$ (311.3) calculated: 65.58% C, 5.50% H, 13.50% N; found: 65.47% C, 5.56% H, 13.71% N. IR: 1665 (C=O), 1609 (CH=CH). 1H NMR: 7.93 d, 1 H, J = 16.0 (H-2); 8.29 d, 1 H, J = 16.0 (H-3); 9.28 d, 1 H, J = 1.4 (H-3'); 8.54 d, 1 H, J = 1.4 (H-6'); 7.85 d, 2 H, J = 8.8 (H-2'' and H-6''); 8.27 d, 2 H, J = 8.8 (H-3'' and H-5''); 0.96 t, 3 H, J = 7.4, 1.42 m, 2 H, 1.78 m, 2 H, 2.93 t, 2 H, J = 7.7 (butyl). ^{13}C NMR: 188.1 (C-1); 124.2 (C-2); 141.7 (C-3); 145.5 (C-2'); 144.2 (C-3'); 161.9 (C-5'); 142.9 (C-6'); 140.9 (C-1''); 129.3 (C-2'' and C-6''); 124.2 (C-3'' and C-5''); 148.6 (C-4''); 13.8, 22.4, 31.4, 35.6 (butyl).

(*E*)-3-(4-Nitrophenyl)-1-(5-propylpyrazin-2-yl)prop-2-en-1-one (**9e**). Yield 40%, m.p. 124–127 °C. For $C_{16}H_{15}N_3O_3$ (297.3) calculated: 64.64% C, 5.09% H, 14.13% N; found: 64.66% C, 5.12% H, 14.09% N. IR: 1672 (C=O), 1612 (CH=CH). 1H NMR: 7.94 d, 1 H, J = 16.0 (H-2); 8.29 d, 1 H, J = 16.0 (H-3); 9.29 d, 1 H, J = 1.4 (H-3'); 8.54 d, 1 H, J = 1.4 (H-6'); 7.85 d, 2 H, J = 8.8 (H-2'' and H-6''); 8.27 d, 2 H, J = 8.8 (H-3'' and H-5''); 1.01 t, 3 H, J = 7.4, 1.83 m, 2 H, 2.91 t, 2 H, J = 7.7 (propyl). ^{13}C NMR: 188.1 (C-1); 124.2 (C-2); 141.7 (C-3); 145.6 (C-2'); 144.3 (C-3'); 161.7 (C-5'); 142.9 (C-6'); 140.9 (C-1''); 129.3 (C-2'' and C-6''); 124.2 (C-3'' and C-5''); 148.6 (C-4''); 14.0, 22.8, 38.0 (propyl).

Evaluation of in vitro Antifungal Activity

Antifungal activity of all compounds against *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E 28, *Candida glabrata* 20/I, *Trichosporon beigeli* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272 and *Trichophyton mentagrophytes* 445 was evaluated by the microdilution broth method. All strains were subcultured on Sabouraud dextrose agar (SDA, Difco) and maintained on the same medium at 4 °C. Prior to testing, each strain was passaged onto SDA and fungal inocula were prepared by suspending yeasts or conidia or sporangiospores in sterile 0.85% saline. The cell density was adjusted, using the Bürker's chamber, to yield a stock suspension of $(1.0 \pm 0.2) \times 10^5$ CFU/ml. The final inoculum was made by 1:20 dilution of the stock suspension with the test medium.

The compounds were dissolved in dimethyl sulfoxide (DMSO) and antifungal activity was determined in the tissue culture medium RPMI 1640 (Sevapharma, Prague, Czech Republic) buffered to pH 7.0 with 0.165 M 3-morpholinopropane-1-sulfonic acid (Sigma). Controls were included as well. The final concentration of DMSO in the test medium did not exceed 1% (v/v) of the total solution composition. The minimum inhibitory concentration (MIC), defined as 80% inhibition of fungal growth compared to control, were determined after 24 and 48 h of static incubation at 35 °C. In the case of *T. mentagrophytes* the MICs were recorded after 72 and 120 h. Ketoconazole (batch No. E 3401, Janssen Pharmaceutica) was used as a reference antifungal drug. The results are given in Table I.

Evaluation of Antimycobacterial Activity

Primary screening of all compounds was conducted at 6.25 µg/ml against *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294) in the Bactec 12B medium using a broth microdilution assay (Microplate Alamar Blue Assay, MABA)⁴⁷. Compounds exhibiting fluorescence were tested in the Bactec 460 radiometric system⁴⁷. The compounds displaying at least 90% inhibition in the primary screen were reevaluated at lower concentrations against *M. tuberculosis* H₃₇Rv to determine the minimum inhibitory concentration (MIC) using MABA. The MIC is defined as the lowest concentration effecting the 90% reduction in fluorescence relative to control. For the results, see Table I.

Evaluation of Cytotoxicity

The compounds that exhibited promising antimycobacterial activity (**7a**, **9a** and **9b**) were tested for cytotoxicity (IC₅₀) in VERO cells at concentrations less than or equal to 10 times the MIC for *M. tuberculosis* H₃₇Rv. After 72-h exposure, viability was assessed on the basis of cellular conversion of 1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. For compound **7a**, IC₅₀ was 0.86 µg/ml and IC₅₀ values for the remaining two derivatives could not be determined due to solubility problems.

Evaluation of Photosynthesis-Inhibiting Activity in Chloroplasts

The oxygen evolution rate (OER) in spinach chloroplasts (chlorophyll content 30 mg/l) was determined spectrophotometrically (Specord UV VIS, Zeiss Jena, Germany) in the presence of 2,6-dichlorophenolindophenol as an electron acceptor using the method described⁴⁸. Due to low water solubility the studied compounds were dissolved in DMSO so that the applied DMSO concentration (up to 5%, v/v) did not affect oxygen evolution. The inhibitory activity was expressed by IC₅₀ values, i.e. concentrations causing 50% OER reduction relative to untreated controls. The results are given in Table I.

Evaluation of Inhibition of Chlorophyll Content in *Chlorella vulgaris*

The algae *Chlorella vulgaris* were statically cultivated (7 days, photoperiod 16 h light/8 h dark; irradiance 80 µmol/m²/s²; pH 7.2) at room temperature according to ref.⁴⁹ Chlorophyll content in the algal suspension was determined spectrophotometrically after extraction with ethanol according to Wellburn⁵⁰. At the beginning of the cultivation, the chlorophyll content in the suspensions was 0.1 mg/l. The tested compounds were dissolved in DMSO, the concentration of which in the algal suspensions did not exceed 0.5% (v/v). The control samples contained the same DMSO amount as the suspensions treated with the tested compounds. For the results, see Table I.

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REFERENCES

1. Bohm B. A. in: *The Flavonoids* (J. B. Harborne, T. J. Mabry and H. Mabry, Eds), 1st ed., p. 442. Chapman and Hall, London 1975.
2. Prokešová M., Rihs G., Toma Š.: *Collect. Czech. Chem. Commun.* **1996**, *61*, 364.
3. Špringerová J., Kačer P., Hájek J., Červený L.: *Collect. Czech. Chem. Commun.* **2003**, *68*, 1927.
4. Chebanov V. A., Muravyova E. A., Sadchikova Y. V., Desenko S. M., Chernenko V. N., Kobzar K. M., Musatov V. I.: *Collect. Czech. Chem. Commun.* **2004**, *69*, 897.
5. Chebanov V. A., Saraev V. E., Gura E. A., Desenko S. M., Musatov V. I.: *Collect. Czech. Chem. Commun.* **2005**, *70*, 350.
6. Goff D. A.: *Tetrahedron Lett.* **1999**, *40*, 8741.
7. Opletalová V.: *Cesk. Slov. Farm.* **1995**, *44*, 305.
8. Baatout S., Derradji H., Jacquet P., Mergeay M.: *Int. J. Mol. Med.* **2005**, *15*, 337.
9. Selvam C., Jachak S. M., Thilagavathi R., Chakraborti A. K.: *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1793.
10. Murias M., Jager W., Handler N., Erker T., Horvath Z., Szekeres T., Nohl H., Gille L.: *Biochem. Pharmacol.* **2005**, *69*, 903.
11. Dimmock J. R., Elias D. W., Beazely M. A., Kandepu N. M.: *Curr. Med. Chem.* **1999**, *6*, 1125.
12. Opletalová V., Šedivý D.: *Cesk. Slov. Farm.* **1999**, *48*, 252.
13. Opletalová V., Řičířáková P., Šedivý D., Meltrová D., Křiváková J.: *Folia Pharm. Univ. Carol.* **2000**, *25*, 21.
14. Opletalová V.: *Cesk. Slov. Farm.* **2000**, *49*, 278.
15. Opletalová V., Jahodář L., Jun D., Opletal L.: *Cesk. Slov. Farm.* **2003**, *52*, 12.
16. Chlupáčová M., Opletalová V.: *Chem. Listy* **2004**, *98*, 320.
17. Stevens J. F., Page J. E.: *Phytochemistry* **2004**, *65*, 1317.
18. Ni L. M., Meng C. Q., Sikorski J. A.: *Exp. Opin. Ther. Pat.* **2004**, *14*, 1669.
19. Go M. L., Lu X., Liu X. L.: *Curr. Med. Chem.* **2005**, *12*, 483.
20. Botta B., Vitali A., Menendez P., Misiti D., Delle Monache G.: *Curr. Med. Chem.* **2005**, *12*, 717.
21. Won S. J., Liu C. T., Tsao L. T., Weng J. R., Ko H. H., Wang J. P., Lin C. N.: *Eur. J. Med. Chem.* **2005**, *40*, 103.
22. Waisser K., Peřina M., Klimešová V., Kaustová J.: *Collect. Czech. Chem. Commun.* **2003**, *68*, 1275.
23. Okunade A. L., Elvin-Lewis M. P. F., Lewis W. H.: *Phytochemistry* **2004**, *65*, 1017.
24. Tripathi R. P., Tewari N., Dwivedi N., Tiwari V. K.: *Med. Res. Rev.* **2005**, *25*, 93.
25. Doležal M.: *Cesk. Slov. Farm.* **2002**, *51*, 226.
26. Pravda M., Kubicová L., Veselý O., Šustr M., Buchta V.: *Folia Pharm. Univ. Carol.* **2004**, *31/32*, 67.
27. Opletalová V., Hartl J., Patel A., Palát K., Jr., Buchta V.: *Farmaco* **2002**, *57* 135.
28. Opletalová V., Hartl J., Palát K., Jr., Patel A.: *J. Pharm. Biomed. Anal.* **2000**, *23*, 55.
29. Chlupáčová M., Kubanová P., Opletalová V., Buchta V.: *Natural Drugs 2003. Proc. 3rd Int. Symp., Naples, October 2–4, 2003* (F. Borelli, F. Capasso, N. Milic and A. Russo, Eds), p. 133. Universita degli Studi di Napoli Federico II, Naples and Indena, Milano 2005.
30. Chlupáčová M., Opletalová V., Kuneš J., Kráľová K.: *Folia Pharm. Univ. Carol.* **2004**, *31/32*, 109.

31. Zámocká J., Dvořáčková D., Heger J.: *Z. Chem.* **1980**, 20, 29.
32. Horák M., Papoušek D.: *Infračervená spektra a struktura molekul*, 1st ed., p. 584. Academia, Praha 1976.
33. Lin Y. M., Zhou Y., Flavin M. T., Zhou L. M., Nie W., Chen F. C.: *Bioorg. Med. Chem.* **2002**, 10, 2795.
34. López S. N., Castelli M. V., Zacchino S. A., Domínguez J. N., Lobo G., Charris-Charris J., Cortés J. C. G., Ribas J. C., Devia C., Rodríguez A. M., Enriz R. D.: *Bioorg. Med. Chem.* **2001**, 9, 1999.
35. Boeck P., Leal P. C., Yunes R. A., Filho V. C., López S., Sortino M., Escalante A., Furlán R. L. E., Zacchino S.: *Arch. Pharm. Chem. Life Sci.* **2005**, 338, 87.
36. Opletalová V., Chlupáčová M., Buchta V., Silva L.: *Natural Drugs 2003. Proc. 3rd Int. Symp., Naples, October 2–4, 2003* (F. Borelli, F. Capasso, N. Milic and A. Russo, Eds), p. 259. Università degli Studi di Napoli Federico II, Naples and Indena, Milano 2005.
37. Kráľová K., Šeršeň F., Opletalová V.: *Folia Pharm. Univ. Carol.* **1998**, 23 (Suppl.), 71.
38. Tsuchiya H., Sato M., Akagiri M., Takagi N., Tanako T., Iinuma M.: *Pharmazie* **1994**, 49, 756.
39. DalPozzo A., Dansi A.: *Boll. Chim. Farm.* **1979**, 118, 239.
40. Kuhn R., Hensel H. R.: *Chem. Ber.* **1953**, 86, 1333.
41. Medvecký R., Ďurinda J., Odlerová Ž., Polášek E.: *Farm. Obzor* **1992**, 61, 341.
42. Kozmík V., Lhoták P., Odlerová Ž., Paleček J.: *Cesk. Slov. Farm.* **1998**, 47, 870.
43. Doshi R., Kagthara P., Parekh H.: *Indian J. Chem., Sect. B.: Org. Chem. Incl. Med. Chem.* **1999**, 38, 348.
44. Friis-Møller A., Chen M., Fuursted K., Christensen S. B., Kharazmi A.: *Planta Med.* **2002**, 68, 416.
45. Lipinski C. A., Lombardo F., Dominy B. W., Feeney P. J.: *Adv. Drug Delivery Rev.* **1997**, 23, 3.
46. Opletalová V., Patel A., Boulton M., Dundrová A., Lacinová E., Převorová M., Appeltauerová M., Coufalová M.: *Collect. Czech. Chem. Commun.* **1996**, 61, 1093.
47. Collins L., Franzblau S. G.: *Antimicrob. Agents Chemother.* **1997**, 41 1004.
48. Kráľová K., Šeršeň F., Sidóová E.: *Chem. Pap.* **1992**, 46, 348.
49. Kráľová K., Šeršeň F., Melník M.: *J. Trace Microprobe Tech.* **1998**, 16 491.
50. Wellburn A. R.: *J. Plant Physiol.* **1994**, 144, 307.
51. Carpentier R., Fuerst E. P., Nakatani H. Y., Arntzen C. J.: *Biochim. Biophys. Acta* **1985**, 808, 293.
52. Fairchild J. F., Ruessler D. S., Carlson A. R.: *Environ. Toxicol. Chem.* **1998**, 17, 1830.