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Ring substituted 3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones as potential photosynthesis-inhibiting, antifungal and antimycobacterial agents

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Abstract

Four series of ring substituted (*E*)-3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones were prepared by means of modified Claisen–Schmidt condensation of acetylpyrazines with aromatic aldehydes. The structures were confirmed by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectra. The compounds were tested for specific biological properties and some derivatives exhibited photosynthesis-inhibiting, antifungal and antimycobacterial properties. The most pronounced effects were observed with compounds substituted with phenolic groups. *Ortho*-hydroxyl substituted derivatives were more potent than the corresponding *para*-hydroxyl substituted analogues. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Ring substituted (*E*)-3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones; Photosynthesis-inhibiting activity; Antifungal activity; Antimycobacterial activity

1. Introduction

Chalcones are natural products that may be used as lead structures for the development of new drugs. Biological activities of chalcones and their heterocyclic analogues have been reviewed recently [1–3]. Four series of ring substituted (*E*)-3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones **1a**–**1e**, **2a**–**2e**, **3a**–**3e** and **4a**–**4e** were prepared as a part of our continuing effort aimed at the synthesis of biologically active pyrazine derivatives.

Conformational analysis of the *ortho*-hydroxyl substituted derivatives **2a**–**2e** based on spectral data and molecular model calculations is available [4]. The present study focuses on the synthetic and biological results. Three types of bioassays were chosen based on the following rationale: pyrazine derivatives [5–7] and cinnamic acid [8] which have structural features in common with the cinnamoylpyrazines in this study are known to influence plant growth, antifungal properties of chalcones and their heterocyclic analogues are well documented in literature [2], and antimycobacterial properties of chalcones and azachalcones have been known since the 1950's [9] and analogous derivatives have recently been shown to be potential antituberculous agents [10–12]. The effect on plant growth was monitored by quantifying the chlorophyll and oxygen evolution rate (OER), as an indirect measure of the effect on photosynthesis. To test the antifungal potential, eight pathogenic strains of fungi were included in the programme of study. In recent years, tuberculosis has again become a problem throughout the world due to the increasing migration from developing countries and the spread of the human immunodeficiency virus infection. Furthermore, the built-up of resistance to the antituberculous drugs by *Mycobacterium tuberculosis* strains and non-tuberculous mycobacterial infections

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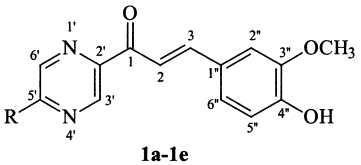
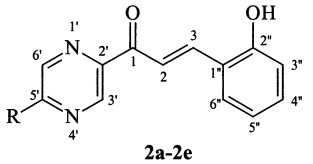


1a-1e



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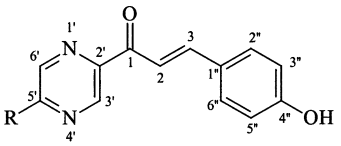

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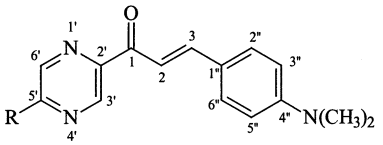
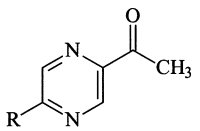


4a-4e

1-5	R
a	H
b	$(CH_3)_3C$
c	$(CH_3)_2CHCH_2$
d	$CH_3CH_2CH_2CH_2$
e	$CH_3CH_2CH_2$



5a-5e

has made the classic armamentarium of antituberculous drugs inefficacious and, thus, the research for new drugs has become a public health priority [13,14]. In 1994, the National Institute of Allergy and Infectious Diseases founded a drug acquisition and screening programme, the TAACF, to facilitate the development, testing, and commercialization of new antituberculous agents. The compounds reported here were screened for the antimycobacterial properties as part of the TAACF project.

2. Experimental

Acetylpyrazines **5a**–**5e** prepared as described previously [15] and commercially available aromatic aldehydes were used as starting materials. Silpearl (Kavalier, Votice) was used for flash column chromatography. Purity of the products was checked by TLC on Silufol UV 254 plates (Kavalier, Votice). The following solvent mixtures were used for TLC: $C_6H_5CH_3$–C_3H_6O 50:50 (v/v), light petroleum–EtOAc 60:40 (v/v), and light petroleum–EtOAc 80:20 (v/v). M.p. were determined with a Boëtius apparatus and are uncorrected. Elemental analyses were performed with CHN Analyzer (Laboratoní přístroje, Prague). IR spectra were recorded in KBr pellets with an IR spectrophotometer Nicolet Impact 400. Characteristic wavenumbers are given in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded at room temperature (r.t.) in 5 mm tubes using a JOEL GSX-270 (^1H, ^{13}C) FT spectrometer at 270.16 (^1H) and 67.97 (^{13}C) MHz, using the deuterium signal of the solvent as the lock and TMS as internal standard. The parameters were: Spectral width 3 (^1H) and 18 kHz (^{13}C), pulse width 3 (^1H) and 4.2 μs (^{13}C), acquisition time 5.46 or 0.90 s, number of scans 16 (^1H) and 600 (^{13}C). Chemical shifts are given in δ, ppm and coupling constants J in Hz. Initial geometries of the molecular models were built by means of standard parameters and then optimized by semi-empirical AM1 method using Polak–Ribiere geometrical optimization, RMS gradient 0.05 kcal $Å^{-1}$ mol^{-1}. Molecular dynamics (with heating to 1200 K) in combination with manual rotation on single bonds was used for finding the conformer with the lowest energy. Other conformers were formed by the manual rotation of the appropriate bonds in the lowest energy model followed by the geometry optimization. Relative ^1H NMR shifts were calculated by semi-empirical TNDO/2 method with special Slater exponents for shielding. HyperNMR software (version 2.0, Hypercube Inc.) was used for the calculation of NMR spectra and the program HyperChem (release 5.11, HyperCube Inc.) for other quantum-chemical calculations. Specord UV–Vis (Zeiss Jena, Germany) was used in photosynthesis-inhibiting bioassays.

2.1. Chemistry

2.1.1. General procedure for the preparation of compounds *1a–1e*, *2a–2e*, *3a–3e* and *4a–4e*

Acetylpyrazine (0.01 mol) and aromatic aldehyde (0.01 mol) were dissolved in Py (4.4 ml). Diethylamine (0.73 g, 0.01 mol) was added and the reaction mixture was heated in the $C_3H_8O_3$ bath at 80–120 °C for 2 h. After cooling, the reaction mixture was poured into ice water acidified to pH 3 with a few drops of AcOH, and

then refrigerated for 24 h. If the product separated as a solid, it was filtered off and recrystallized several times from absolute EtOH. If the product separated as an oily mixture, it was extracted with Et_2O and subjected to the flash column chromatography on silica gel using the mixture light petroleum–EtOAc 60:40 (v/v) as an

Table 1
Characteristic data and IR spectra of compounds **1**, **2**, **3** and **4**

Comp.	M.p. (°C) Yield (%)	Formula M.w.	ν (cm^{-1})		
			C=O *s-cis*	C=O *s-trans*	E–C(O)CH=CH
1a	182–184 [a] 23	$C_{14}H_{22}N_2O_3$ 256.3	1664	1637	990
1b	151–152 33	$C_{18}H_{20}N_2O_3$ 321.4	1667	N	985
1c	143–145 25	$C_{18}H_{20}N_2O_3$ 321.4	1664	N	987
1d	148–150 8	$C_{18}H_{20}N_2O_3$ 321.4	1664	1637	987
1e	182–185.5 8	$C_{17}H_{18}N_2O_3$ 298.3	1664	1636	1009
2a	172.5–174.5 [b] 35	$C_{13}H_{10}N_2O_2$ 226.2	1665	1636	992
2b	182–185.5 35	$C_{17}H_{18}N_2O_2$ 282.3	1652	1630	993
2c	152–156 28	$C_{17}H_{18}N_2O_2$ 282.3	1652	N	997
2d	132–134 36	$C_{17}H_{18}N_2O_2$ 282.3	1661	1637	987
2e	148–150.5 26	$C_{16}H_{16}N_2O_2$ 268.3	1651	N	998
3a	188–190 [c] 44	$C_{13}H_{10}N_2O_2$ 226.2	1674	1636	986
3b	182–185.5 46	$C_{17}H_{18}N_2O_2$ 282.3	1663	1637	987
3c	151–153.5 35	$C_{17}H_{18}N_2O_2$ 282.3	1665	N	989
3d	126–127 36	$C_{17}H_{18}N_2O_2$ 282.3	1663	N	1002
3e	135–137 37	$C_{16}H_{16}N_2O_2$ 268.3	1664	N	1010
4a	162–165 20	$C_{15}H_{15}N_3O$ 253.3	1658	1635	994
4b	108–118 10	$C_{19}H_{23}N_3O$ 309.4	1651	N	996
4c	78–81 7	$C_{19}H_{23}N_3O$ 309.4	1656	1636	1003
4d	90–92 10	$C_{19}H_{23}N_3O$ 309.4	1652	1629	990
4e	128.5–130.5 44	$C_{18}H_{21}N_3O$ 295.4	1652	N	992

N, not observed.
[a] Ref. [23] 183–185 °C.
[b] Ref. [23] 173–175 °C.
[c] Ref. [23] 191–194 °C.

Table 2
^{1}H NMR spectra of compounds **1**, **2**, **3**, and **4**

Comp.	Chemical shifts (δ (ppm); J (Hz))										Substituents
	H-2	H-3	H-3′	H-5′	H-6′	H-2″	H-3″	H-4″	H-5″	H-6″	
1a [a]	8.00 d 1H $J = 15.8$	7.90 d 1H $J = 15.8$	9.33 d 1H $J = 1.5$	8.78 d 1H $J = 2.6$	8.72 dd 1H $J = 1.5$ and 2.6	7.22 d 1H $J = 1.8$			6.94 d 1H $J = 8.6$	7.23 dd 1H $J = 1.8$ and 8.6	3.96s, 3H(OCH_3) 8.85s, 1H(OH)
2a [b]	8.24 d 1H $J = 16.1$	8.31 d 1H $J = 16.1$	9.31 d 1H $J = 1.5$	8.78 d 1H $J = 2.6$	8.72 dd 1H $J = 1.5$ and 2.6		6.95 dd 1H $J = .0.9$ and 7.9	7.25 m 1H $J = 1.6$, 7.9 and 7.9	6.87 m 1H $J = 0.9$, 7.9 and 7.9	7.66 dd 1H $J = 1.6$ and 7.9	9.83s, 1H(OH)
3a [c]	8.00 d 1H $J = 15.9$	7.90 d 1H $J = 15.9$	9.31 d 1H $J = 1.5$	8.78 d 1H $J = 2.6$	8.72 dd 1H $J = 1.5$ and 2.6	7.61 dd 2H $J = 4.6$ and 8.6	6.89 dd 2H $J = 4.6$ and 8.6		6.89 dd 2H $J = 4.6$ and 8.6	7.61 dd 2H $J = 4.6$ and 8.6	9.73s, 1H(OH)
4a [d]	7.95s 2H	7.95s 2H	9.36 d 1H $J = 1.5$	8.72 d 1H $J = 2.6$	8.66 dd 1H $J = 1.5$ and 2.6	7.62 dd 2H $J = 4.0$ and 9.0	6.67 dd 2H $J = 4.0$ and 9.0		6.67 dd 2H $J = 4.0$ and 9.0	7.62 dd 2H $J = 4.0$ and 9.0	3.04s, 6H($N(CH_3)_2$)
4a [e]	8.09 d [g] 1H $J = 15.8$	7.95 d [g] 1H $J = 15.8$									
4a [f]	8.37 d [g] 1H $J = 15.8$	8.40 d [g] 1H $J = 15.8$	9.63 d 1H $J = 1.5$	8.06 d 1H $J = 2.6$	7.93 dd 1H $J = 1.5$ and 2.6	7.46 dd 2H $J = 4.0$ and 9.0	6.29 dd 2H $J = 4.0$ and 9.0		6.29 dd 2H $J = 4.0$ and 9.0	7.46 dd 2H $J = 4.0$ and 9.0	2.32s, 6H($N(CH_3)_2$)

[a] Signal for H-5′ was missing in the spectra of alkylated derivatives **1b**–**1e**. Chemical shifts for alkyl groups were: 1.45s, 9H, ((CH_3)$_3$C); 0.95d, 6H, $J = 6.6$, ((CH_3)$_2$$CHCH_2$), 2.18m, 1H, $J = 6.6$ and 7.2 ((CH_3)$_2$$CHCH_2$), 2.79d, 2H, $J = 7.2$ ((CH_3)$_2$$CHCH_2$); 0.97t, 3H, $J = 7.3$ ($CH_3CH_2CH_2CH_2$), 1.42m, 2H, $J = 7.3$ and 7.3 ($CH_3CH_2CH_2CH_2$), 1.78m, 2H, $J = 7.3$ and 7.7 ($CH_3CH_2CH_2CH_2$), 2.92t, 2H, $J = 7.7$ ($CH_3CH_2CH_2CH_2$); 1.02t, 3H, $J = 7.5$ ($CH_3CH_2CH_2$), 1.83m, 2H, $J = 7.5$ and 7.3 ($CH_3CH_2CH_2$), 2.91t, 2H, $J = 7.3$ ($CH_3CH_2CH_2$). The remaining chemical shifts and coupling constants were close to that of **1a** ($\pm$ 0.06 ppm/0.4 Hz) with exception of H-6′ in **1c**, **1d** and **1e** that were about 8.53 ppm, and OH groups that were 6.16 for **1b**, 6.28 for **1c**, 6.03 for **1d** and 7.60 for **1e**.

[b] Signal for H-5′ was missing in the spectra of alkylated derivatives **2b**–**2e**. Chemical shifts for alkyl groups were practically the same as in the series **1**. The remaining chemical shifts and coupling constants were close to that of **2a** ($\pm$ 0.10 ppm/0.0 Hz), H-6′ in **1c**, **1d** and **1e** were about 8.53 ppm, and OH groups were 9.57 for **2b**, 9.65 for **2c**, 9.70 for **2d** and 9.74 for **2e**.

[c] Signal for H-5′ was missing in the spectra of alkylated derivatives **3b**–**3e**. Chemical shifts for alkyl groups were practically the same as in the series **1**. The remaining chemical shifts and coupling constants were close to that of **3a** ($\pm$ 0.09 ppm/0.0 Hz), H-6′ in **1c**, **1d** and **1e** were about 8.53 ppm, and OH groups were 9.62 for **3b**, 9.69 for **3c**, 9.60 for **3d** and 9.62 for **3e**.

[d] Signal for H-5′ was missing in the spectra of alkylated derivatives **4b**–**4e**. Chemical shifts for alkyl groups were practically the same as in the series **1**. The remaining chemical shifts (including the signal for dimethylamino group) and coupling constants were close to that of **4a** ($\pm$ 0.02 ppm/0.0 Hz), H-6′ in **4c**, **4d** and **4e** were about 8.50 ppm.

[e] Sample diluted 50-fold with $CDC1_3$ before obtaining spectrum, remaining signals were slightly broad and are not reported here.

[f] Spectrum obtained in C_6D_6.

[g] Assignments are reversible.

Table 3
^{13}C NMR spectra of compounds **1**, **2**, **3**, and **4**

Comp.	Chemical shifts (δ (ppm))													Substituents
	C-1	C-2	C-3	C-2′	C-3′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	
1a [a]	188.2	116.9	146.4	150.2	144.5	147.3	143.4	126.6	110.8	147.9 [c]	148.7 [c]	115.7	124.4	56.0 (OCH_3)
2a [b]	188.7	119.5	141.6	148.7	144.4	147.2	143.4	121.7	148.7	116.5	132.2	119.5	129.1	
3a [b]	188.2	116.6	146.0	148.6	144.3	147.3	143.4	125.9	130.7	116.2	160.8	116.2	130.7	
4a [b]	188.1	114.5	146.3	149.3	144.7	146.9	143.2	122.5	131.1	111.7	152.3	111.7	131.1	40.1 ($N(CH_3)_2$)

[a] Chemical shifts for alkyl groups were: 29.7 (($\mathbf{C}H_3)_3C$), 37.1 (($CH_3)_3\mathbf{C}$); 22.4 (($\mathbf{C}H_3)_2CHCH_2$), 29.1 (($CH_3)_2\mathbf{C}HCH_2$), 44.7 (($CH_3)_2CH\mathbf{C}H_2$); 13.8 ($\mathbf{C}H_3CH_2CH_2CH_2$), 22.4 ($CH_3\mathbf{C}H_2CH_2CH_2$), 31.5 ($CH_3CH_2\mathbf{C}H_2CH_2$), 35.5 ($CH_3CH_2CH_2\mathbf{C}H_2$); 13.7 ($\mathbf{C}H_3CH_2CH_2$), 22.4 ($CH_3\mathbf{C}H_2CH_2$), 37.5 ($CH_3CH_2\mathbf{C}H_2$). The remaining values were close to that of **1a** ($\pm$4.2 ppm) with exception of C-5′ that were shifted slightly downfield, i.e. 167.5 for **1b**, 160.3 for **1c**, 161.2 for **1d** and 160.7 for **1d**.

[b] Chemical shifts for alkylated compounds showed a pattern similar to that of alkylated derivatives of series **1**.

[c] Assignments are reversible.

Table 4
Photosynthesis-inhibiting properties of compounds **2b**–**2e** and **3b**–**3e**

Comp.	Inhibition of chlorophyll production in *C. vulgaris* at 0.1 mmol l^{-1} (%)	Inhibition of oxygen evolution rate IC_{50} (mmol l^{-1})	
		Spinach chloroplasts	*C. vulgaris*
2b	27	0.167	0.078
2c	92	0.144	0.063
2d	89	0.184	0.147
2e	68	0.187	0.100
3b	39	0.315	0.279
3c	35	0.235	0.232
3d	10	0.306	0.265
3e	12	0.399	0.514
KlimCS-8	100 [a]	0.072 [b]	0.048 [c]
Dol-2	N	0.105 [d]	0.099 [d]
Dol-5	N	0.478 [d]	0.329 [d]

[a] Ref. [34], KlimCS-8 = 2-octylthio-4-pyridinecarbothioamide.

[b] Ref. [29].

[c] Ref. [35].

[d] Ref. [36], Dol-2 = 5-*tert*-butyl-6-chloro-*N*-(3-chloro-5-hydroxyphenyl)-2-pyrazinecarboxamide, Dol-5 = 5-*tert*-butyl-*N*-(3-hydroxyphenyl)-2-pyrazinecarboxamide.

N, not determined.

eluent. After separation the product was crystallized from absolute EtOH. The microanalyses (C, H, N) were within $\pm$0.4% for all new compounds. IR spectra of the products are given in Table 1 and the NMR data in Tables 2 and 3.

2.2. Biological evaluation

2.2.1. Evaluation of photosynthesis-inhibiting activity

The compounds were dissolved in dimethyl sulfoxide (DMSO) rather than water due to their restricted solubility in the latter solvent. DMSO concentration in the samples was found not to affect the photochemical activity of spinach chloroplasts and algal suspensions.

The algae, *Chlorella vulgaris*, were statically cultivated (photoperiod 16 h light/8 h dark; illumination: 60 μEs^{-1} m^{-2} PAR) at r.t. according to the method described in Ref. [16]. The samples contained 0.1 mmol l^{-1} of the studied compounds and the resulting solvent concentration in the samples as well as in the control was adjusted to 1%. Seven days after addition of the test compounds, chlorophyll content in the algal suspensions was determined spectrophotometrically after extraction into *N*,*N* dimethylformamide [17].

The OER in spinach chloroplasts ($C_{chloroplasts}$ = 30 mg l^{-1}) was determined spectrophotometrically using 2,6-dichlorophenol–indophenol as an electron acceptor according to the method reported previously [18].

OER in the algal suspension of *C. vulgaris* ($C_{chlorophyll}$ = 20 mg l^{-1}) was measured at 24 °C by Clark type electrode (SOPS 31 atp., Chemoprojekt, Prague) in a chamber constructed according to a reported method [19]. The suspensions were accommodated in the dark (4 h) prior to the OER

measurements. The samples were then illuminated with a 250 W halogen lamp from 0.3 m distance through a water filter (400 μEs^{-1} m^{-2} PAR) at r.t. [20]. The results are summarized in Table 4.

2.2.2. Evaluation of in vitro antifungal activity

Antifungal susceptibility of all the compounds was assessed by microdilution broth method against *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E 28, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigates* 231 and *Absidia corymbifera* 272. All strains were subcultured on Sabouraud dextrose agar (SDA) 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 by means of a Bürker's chamber to yield a stock suspension of 1.0–5.0×10^5 cfu ml^{-1}. The final inoculum was made by 1:20 dilution of the stock suspension with the test medium.

Antifungal activity of the compounds **1a**–**1e**, **2a**–**2e**, **3a**–**3e** and **4a**–**4e**, dissolved in DMSO, was determined in tissue culture medium RPMI 1640 (Sevac) buffered to pH 7.0 with 0.165 M 3-(*N*-morpholino)propanesulfonic acid (Sigma). Controls without the test compounds were also included. The minimum inhibitory concentrations (MIC), defined as the 80% inhibition of fungal growth compared to control wells, were

Table 5
Inhibitory activity of compounds **1**, **2**, **3** and **4** against *T. mentagrophytes* 445 and *M. tuberculosis* $H_{37}Rv$

Comp.	Inhibition of *T. mentagrophytes* 445 MIC ($\mu mol\ l^{-1}$)		Inhibition of *M. tuberculosis* $H_{37}Rv$ at 12.5 $\mu g\ ml^{-1}$ (%)
1a	125	N	35
1b	>125	N	77
1c	250	N	53
1d	>100	N	45
1e	>125	N	20
2a	15.62	31.25	55
2b	>250	>250	94
2c	31.25	N	20
2d	3.91	15.62	33
2e	7.81	7.81	50
3a	62.5	125	42
3b	>250	>250	72
3c	>500	>500	63
3d	31.25	62.5	51
3e	31.25	62.5	35
4a	>62.5	>62.5	26
4b	>250	>250	7
4c	250	>250	10
4d	>250	>250	0
4e	>250	>250	8
pyrazineamide	N	N	100

N, not determined

Table 6
Antifungal activity of compound **2a**

Comp.	MIC ($\mu mol\ l^{-1}$)							
	CA	**CT**	**CK**	**CG**	**TB**	**TM**	**AF**	**AC**
	24 h 48 h	24 h 48 h	24 h 48 h	24 h 48 h	24 h 48 h	72 h 120 h	24 h 48 h	24 h 48 h
2a	62.5 62.5	125 250	125 250	125 250	125 250	15.62 31.25	125 250	125 250
Ketoconazole	≤0.06 ≤0.06	3.91 7.81	1.95 1.95	0.49 1.95	≤0.06 ≤0.06	0.24 1.95	7.81 7.81	15.63 15.63

CA, *Candida albicans* ATCC 44859; **CT**, *Candida tropicalis* 156; **CK**, *Candida krusei* E 28; **CG**, *Candida glabrata* 20/I; **TB**, *Trichosporon beigelii* 1188; **TM**, *Trichophyton mentagrophytes* 445; **AF**, *Aspergillus fumigates* 231; **AC**, *Absidia corymhifera* 272.

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 for this part of the study are given in Tables 5 and 6.

2.2.3. Evaluation of antimycobacterial activity

Primary screening of all the compounds was conducted at 12.5 μg ml^{-1} against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the BACTEC 460-radiometric system (Table 5). Compound **2b** demonstrating 94% inhibition in the primary screen was re-tested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual MIC in a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. This compound was also tested against *M. avium* using the same technique and procedure.

3. Results and discussion

3.1. Chemistry

All the 3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones reported in the present paper are novel with exception of **1a**, **2a** and **3a** that have been reported previously [21–24]. Compounds **1a**, **2a**, **3a** and **4a** were isolated directly as solids since acetylpyrazine **5a** is freely soluble in water and does not contaminate the required products after pouring the reaction mixture into acidified water. In the case of derivatives with an alkylated pyrazine ring (**1b**–**1e**, **2b**–**2e**, **3b**–**3e** and **4b**–**4e**), an oily mixture of the product and unreacted acetylpyrazine resulted. Each compound was then obtained by means of flash column chromatographic separation using silica gel. The products recovered are crystalline, coloured solids; compounds **1a**–**1e**, **2a**–**2e** and **3a**–**3e** are yellow or orange, compounds **4a**–**4e** are red.

Structural confirmation of the products was based on elemental analysis, and IR, ^1H and ^{13}C NMR spectroscopic analysis. IR spectra of all compounds conformed to literature data for analogous structures [23–26]. Carbonyl group can present an *s-cis* or *s-trans* conformation with respect to the vinyl double bond. A conformational equilibrium between the two conformers is dependent on their structures and the properties of environment, e.g. solvent and temperature. A strong absorption band in the region of 1651–1674 cm^{-1} was found in the IR spectra of all the present 3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones, and it was concluded that the more stable *s-cis*-conformer prevailed in the solid state (KBr pellets). A shoulder at 1636 cm^{-1} was observed in the spectra of several compounds. This was taken to indicate the presence of a small amount of the unfavoured *s-trans* conformer [4].

The chemical shift assignments (^1H and ^{13}C NMR) of the four series of compounds were made using DEPT and 2D-Hetero (^1H, ^{13}C) COSY techniques. The magnitude of the olefinic vicinal coupling constant between H-2 and H-3 ($J = 15–16$ Hz) in the ^1H NMR spectra of all the compounds in the series **1**, **2** and **3** is indicative of *E*-configuration. In contrast, the corresponding protons of compounds in the **4** series with the *para*-dimethylamino substituent show a singlet. However, the ^{13}C NMR shifts for C-2 and C-3 are highly comparable to the values obtained for compounds in the series **1**, **2** and **3**. In order to investigate the effect of solute–solvent interactions, the ^1H NMR spectra of **4a** and **4d** were obtained in $CDC1_3$ after a 50-fold dilution. Both the resulting spectra then gave the expected AB quartets ($J_{H2,H3} = 15.8$ Hz). Attempts were also made to obtain spectra of **4a**, **4b** and **4d** in C_6D_6. Sample solubility was limited in each case, but the spectra of the solutions also showed clear AB quartets (**4a**: δ 8.37 and 8.40; **4b**: δ 8.46 and 8.52; **4d**: δ 8.43 and 8.53; $J = 15.8$ Hz).

The relative ^1H NMR chemical shift positions of H-2 and H-3 are reversed for compounds of series **2** on the basis of 2D-Hetero COSY spectra (compared to that of other three series). NOE experiments and molecular model calculations [4] indicated that there is a lack of free rotation of the phenyl ring in the compounds of this series. Experimental ^1H NMR data of compounds **2a**–**2e** were, therefore, compared with those calculated for various conformers of compound **2a** (Fig. 1). The calculated relative ^1H NMR absorptions are δ 7.040 (H-2) and 8.665 (H-3) for conformer *I*, in which H-3 and OH-2″ are in close proximity. In contrast, the calculated relative ^1H NMR signals for H-2 and H-3 are reversed (δ 8.025 and 7.198, respectively) for conformer *II*, in which H-2 is in close proximity to the OH-2″ group. Since the presence of a mixture of (*E*)-*s-cis*- and (*E*)-*s-trans*-conformers was expected in a chloroform solution on the basis of literature data [26], the ^1H NMR shifts were also calculated for the two (*E*)-*s-trans*-conformers *III* and *IV*. For the results see Fig. 1. The observed relative ^1H NMR chemical shifts positions of H-2 and H-3 for compounds of series **2** (proton H-3 further downfield than proton H-2) conform to the calculated values for conformer *I*. According to the results of molecular model calculations reported previously conformer *I* is the most stable one [4]. Similar conformation has been reported for structurally related *ortho*-hydroxyl substituted bis(benzylidene)cycloalkanones [27]. The phenolic protons are probably involved in weak hydrogen bonds (most likely with the solvent or between two or more solute molecules), since their chemical shifts are observed between 9 and 10 ppm.

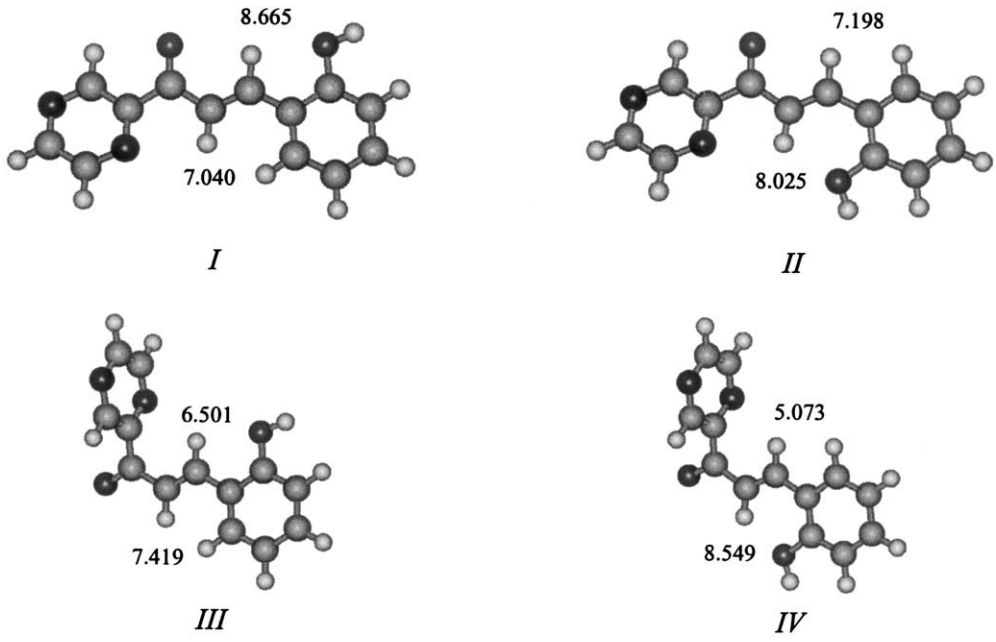


Fig. 1. The relative ^{1}H NMR shift position (in δ, ppm) for conformers of compound **2a** calculated using TNDO/2 method.

3.2. Biological evaluation

With regard to the influence of pyrazine derivatives on plant growth, one study [28] has shown with the aid of EPR spectroscopy that the site of action of 3-phenyl-1-(2-pyrazinyl)-2-propen-1-ones is in the photosynthetic apparatus of spinach chloroplasts. The intensity of the EPR signal II, mainly that of its constituent signal II_{slow}, was decreased. This implied that these compounds interact predominantly with D^+ intermediate, i.e. with the tyrosine radical in position 161 (Tyr_D) which is located in D_2 protein on the donor side of photosystem (PS) 2. Due to this interaction the photosynthetic electron transport from the oxygen evolving complex to the primary donor of PS 2 is impaired and consequently the electron transport between PS 1 and PS 2 is inhibited as well. A similar site of action (Tyr_D) has been confirmed for other inhibitors with a heterocyclic skeleton, for example 2-alkylthio-4-pyridinecarbothioamides [29] and anilides of 2-pyrazinecarboxylic acid [30], whereas the anilides of 2-alkyl-4-pyridinecarboxylic acids are claimed to interact also with the Z^+ intermediate [31]. The effects of the diazachalcones in the present study on statically cultured *C. vulgaris* were found to lower the chlorophyll content (10–92%) by compounds **2b**–**2e** and **3b**–**3e**, compared to untreated controls (Table 4). The compounds of the series **2** and **3** also reduced the rate of oxygen evolution in spinach chloroplasts and *C. vulgaris*. IC_{50} values of these compounds, i.e. concentrations causing 50% inhibition of OER, were compared with IC_{50} values of several compounds known to exhibit the same mechanism of action and are given in Table 4. Photosynthetic electron transport IC_{50} values for some known herbicides can be found in Ref. [32], e.g. for various urea derivatives IC_{50} are in the range 39.81–0.05 μmol l^{-1} and for atrazine between 0.79 and 0.25 μmol l^{-1}. According to Carpentier et al. [33], IC_{50} of atrazine is about 1 μmol l^{-1}. In both types of tests, inhibitory activity of *ortho*-hydroxyl substituted derivatives **2b**–**2e** was greater than that of *para*-hydroxyl substituted ones **3b**–**3e**. The lowest IC_{50} values were found with compounds having a branched alkyl group on the pyrazine ring (**2b**, **2c**).

All the compounds under study were also subjected to an assessment of in vitro antifungal susceptibility. Some compounds with phenolic groups inhibited growth of *T. mentagrophytes* 445. MIC are listed in Table 5. As with the photosynthesis-inhibiting activity the most pronounced antifungal effects were observed with *ortho*-hydroxyl substituted derivatives. This is in accordance with literature data pointing out the importance of hydroxyl groups for antibacterial [3,37] and antifungal [2,38,39] effects of chalcones. In contrast to the observations made with the photosynthesis-inhibiting bioassay, the hydroxyl derivatives with non-branched alkyl substituents on the pyrazine ring (**2d**, **2e**) were most potent. Activity against *Candida* species (MIC = 62.5 μmol l^{-1}) was exhibited by compounds **2d** and **3d**. Compound **2a** showed weak to moderate activity against all testing strains (Table 6).

In the antimycobacterial bioassay, *para*-dimethylamino substituted derivatives (with exception of compound **4a**) were less active than compounds with the phenolic group (series **1**, **2** and **3**). The highest activity was displayed by compounds **1b**, **2b** and **3b**. Thus, the presence of a *tert*-butyl group on the pyrazine nucleus

seems favourable for antimycobacterial activity (Table 5). MIC of the most active derivative **2b** was 12.5 μg ml^{-1}, while rifampicin in the same test was 0.06 μg ml^{-1}. Compound **2b** was also tested against *Mycobacterium avium*, a naturally drug-resistant opportunistic pathogen, and it inhibited the growth of this strain by 32%, MIC > 12.5 μg ml^{-1}. Clarithromycin (MIC = 4 μg ml^{-1}) was used as the positive control against *M. avium*. It is important to note that the most active compound **2b** also belongs to the *ortho*-hydroxyl substituted series. Further studies are underway aimed at the detailed elucidation of structure–activity relationships in the class of chalcones and their analogues to discover a suitable drug candidate.

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