AZACHALCONE DERIVATIVES AND THEIR BIS SUBSTITUTED ANALOGS AS NOVEL ANTIMYCOBACTERIAL AGENTS

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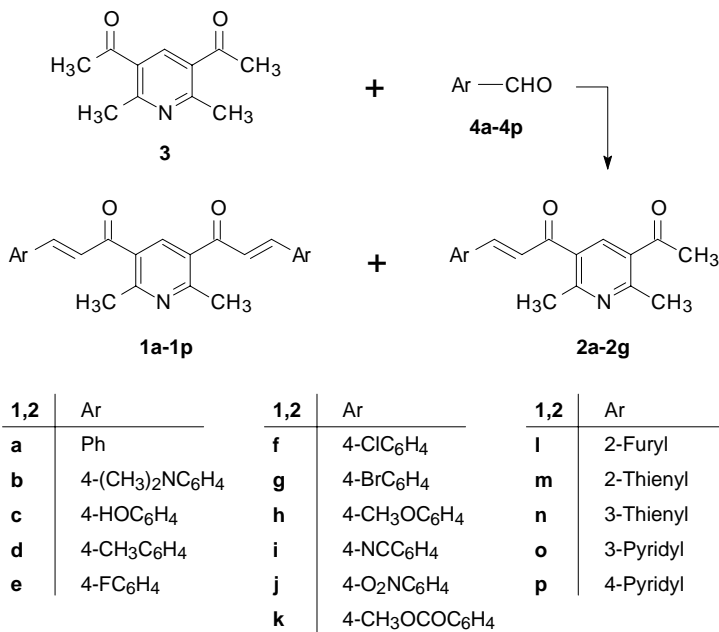[b] *Institute of Preventive and Clinical Medicine, 833 01 Bratislava, Slovak Republic*

Fifteen novel substituted azachalcone derivatives and their bis substituted analogs were prepared from 3,5-diacetyl-2,6-dimethylpyridine by the Claisen–Schmidt condensation. The influence of the reaction conditions on the yield of mono- and bischalcones was studied to optimize the reaction. Spectral characteristics along with preliminary results of antimycobacterial activity of selected compounds are given.

Key words: Azachalcones; Pyridines; 1,1′-(Pyridine-3,5-diyl)bis(3-arylprop-2-en-1-ones); Antimycobacterial activity; Antituberculotics.

It is well known that the patients with immunosuppressive disorders (including AIDS) are highly sensitive towards a broad range of protozoal, fungal, viral and bacterial infections[1,2]. Among these pathogenic microorganisms one can find extremely "multidrug" resistant strains, such as *Mycobacteria tuberculosis* that represents potential danger for the mankind due to the possibility of world-wide expansion of tuberculosis[3–5] noncurable by standard antituberculotics. Recently, many new chalcone derivatives with antiviral[6], antimitotic[7], anti-inflammatory[8], antimalarial[9] and antibacterial[10] activities have been described. Medvecky *et al.*[11] showed azachalcone derivatives exhibiting the same efficiency as a generally accepted standard isoniazid[12] (INH), however, with much higher toxicity. The above-mentioned work[11] together with our own promising results[13] of the testing of **1a** for antimycobacterial activity focused our interest on new synthetic research in the family of substituted azabischalcones. Our aim was to prepare new chalcone derivatives based on the pyridine moiety (Scheme 1, Table I) and evaluate their activity towards various mycobacterial strains.

As we have mentioned in our previous publication[14], the yield of the Claisen–Schmidt condensation (*i.e.*, reaction of methyl aryl ketones with arenaldehydes) strongly depends on the reaction conditions and also on the presence of some substituents in the reactants[14–17].

To optimize the above described condensation, we have focused our interest on a more detailed study of the influence of the reaction conditions (reactant ratio, solvent, catalyst, temperature, reaction time) on the yield of azamonochalcones **2a** and similar bis substituted derivatives **1a**. The course of the reactions was monitored by TLC and the reaction was stopped either after disappearing of 3,5-diacetyl-2,6-dimethylpyridine (**3**) or when polymeric compounds were produced in higher yields.

1,2	Ar	1,2	Ar	1,2	Ar
a	Ph	**f**	4-ClC$_6$H$_4$	**l**	2-Furyl
b	4-(CH$_3$)$_2$NC$_6$H$_4$	**g**	4-BrC$_6$H$_4$	**m**	2-Thienyl
c	4-HOC$_6$H$_4$	**h**	4-CH$_3$OC$_6$H$_4$	**n**	3-Thienyl
d	4-CH$_3$C$_6$H$_4$	**i**	4-NCC$_6$H$_4$	**o**	3-Pyridyl
e	4-FC$_6$H$_4$	**j**	4-O$_2$NC$_6$H$_4$	**p**	4-Pyridyl
		k	4-CH$_3$OCOC$_6$H$_4$		

SCHEME 1

The results are collected in Table II. It is obvious (see Table II, entries 1–3) that the ratio of bischalcone : monochalcone derivatives (**1a** : **2a**) in the acid-catalyzed reaction depends on the amount of sulfuric acid. At a low concentration of H$_2$SO$_4$ in acetic acid (1 : 40, v/v), used as a solvent (Table II, entry 2), the reaction did not occur at all, while a higher concentration of sulfuric acid (2 : 40) led to the isolation of compound **2a** in 58% yield (Table II, entry 1). It is interesting that a slightly higher concentration (3 : 40) gave the bis substituted derivative **1a** (59%) as a main product (Table II, entry 3) while the overall yield of the condensation (**1a** + **2a**) remained approximately the same.

Recently, the base-catalyzed condensation of 2,6-diacetylpyridine with 4-substituted benzaldehydes has been described[18]. Based on these results, we have attempted using various bases, such as KOH, CH$_3$COOK, piperidine, diethylamine and Ba(OH)$_2$, in our reaction system using methanol as a solvent. As follows from Table II, the reaction carried out at low temperature (entry 5) afforded only low yields of products. On the other hand, the same reaction mixture at higher temperature (60 °C) gave mainly

Characteristics of compounds **1**, **2** and **5**

Compound	Yield, % Method	M.p., °C Solvent[a]	Formula M.w.	Calculated/Found		
				% C	% H	% N
1a	74	88–90	$C_{25}H_{21}NO_2$	81.72	5.76	3.81
	A	MeOH	367.4	81.57	5.76	3.81
1b	51	229–232	$C_{29}H_{31}N_3O_2$	76.79	6.89	9.26
	A	Ac–MeOH	453.6	76.64	6.72	9.41
1c	12	215–217	$C_{25}H_{21}NO_4$	75.17	5.30	3.51
	A	Ch–MeOH	399.4	74.78	5.50	3.35
1d	62	122–123	$C_{27}H_{25}NO_2$	82.00	6.37	3.54
	A	MeOH	395.5	81.75	6.52	3.54
1e[b]	70	168–169	$C_{25}H_{19}F_2NO_2$	74.43	4.75	3.47
	A	Ch–MeOH	403.4	74.38	4.97	3.35
1f[c]	68	175–177	$C_{25}H_{19}Cl_2NO_2$	68.82	4.39	3.21
	A	Ch–MeOH	436.3	68.79	4.46	3.11
1g[d]	64	191–193	$C_{25}H_{19}Br_2NO_2$	57.17	3.65	2.67
	A	EA–Hex	525.2	56.87	3.70	2.63
1h	66	130–131	$C_{27}H_{25}NO_4$	75.86	5.89	3.28
	A	Ch–MeOH	427.4	75.72	6.00	3.28
1i	37	230–232	$C_{27}H_{19}N_3O_2$	77.68	4.59	10.07
	A	Ch–MeOH	417.5	77.55	4.72	10.07
1j	12	260–262	$C_{25}H_{19}N_3O_6$	65.64	4.19	9.19
	A	Ch–Py	457.4	65.61	4.21	9.02
1k	16	176–177	$C_{29}H_{25}NO_6$	72.04	5.21	2.90
	A	EA–Hex	483.5	71.84	5.09	2.69
1l	59	130–131	$C_{21}H_{17}NO_4$	72.61	4.93	4.03
	A	EA–Hex	347.4	72.49	4.67	3.87
1m[e]	62	137–139	$C_{21}H_{17}NO_2S_2$	66.46	4.52	3.69
	A	EA–Hex	379.5	67.06	4.57	3.75
1n[f]	46	112–114	$C_{21}H_{17}NO_2S_2$	66.46	4.52	3.69
	A	EA–Hex	379.5	66.64	4.62	3.79

TABLE I
(*Continued*)

Compound	Yield, % Method	M.p., °C Solvent[a]	Formula M.w.	Calculated/Found		
				% C	% H	% N
1o	22	159–160	$C_{23}H_{19}N_3O_2$	74.78	5.18	11.37
	B	Ch–MeOH	369.4	74.55	4.99	11.05
1p	12	166–168	$C_{23}H_{19}N_3O_2$	74.78	5.18	11.37
	B	Ch–MeOH	369.4	74.63	5.08	11.11
2a	2	78–84	$C_{18}H_{17}NO_2$	77.40	6.13	5.01
	A	MeOH	279.3	77.22	6.10	4.72
2b	4	128–130	$C_{20}H_{22}N_2O_2$	74.51	6.88	8.69
	A	EA–Hex	322.4	74.80	6.97	8.75
2c	1	190–195	$C_{18}H_{17}NO_3$	73.20	5.80	4.74
	A	EA–Hex	295.3	72.98	5.90	4.58
2d	5	106–108	$C_{19}H_{19}NO_2$	77.79	6.53	4.77
	A	EA–Hex	293.4	77.80	6.69	4.85
2e[g]	5	105–107	$C_{18}H_{16}FNO_2$	72.71	5.42	4.71
	A	Ch–MeOH	297.3	72.78	5.57	4.65
2f[h]	6	123–125	$C_{18}H_{16}ClNO_2$	68.90	6.14	4.46
	A	Ch–MeOH	313.8	68.65	5.17	4.24
2g[i]	4	135–137	$C_{18}H_{16}BrNO_2$	60.35	4.50	3.91
	A	EA–Hex	358.2	60.03	4.68	4.00
5a	87	85–87	$C_{25}H_{25}NO_2$	80.83	6.78	3.77
		EA–Hex	371.5	80.67	6.59	3.66

[a] Crystallization solvents: MeOH methanol, EA ethyl acetate, Ch chloroform, Hex hexane, Ac acetone; [b] % F calculated: 9.42, found: 9.54; [c] % Cl calculated: 16.25, found: 16.56; [d] % Br calculated: 30.43, found: 30.15; [e] % S calculated: 16.90, found: 16.67; [f] % S calculated: 16.90, found: 16.71; [g] % F calculated: 6.39, found: 6.68; [h] % Cl calculated: 11.30, found: 11.56; [i] % Br calculated: 22.30, found: 22.19.

polymeric products (entries 6, 7). The highest yields of **1a** (70–74%) were achieved with the reactant molar ratio 10 : 40 in methanol at room temperature (entries 4, 8, 9). In all these cases the yield of monoderivative **2a** was very low (<5%). Contrary to our expectations, the condensation accomplished at higher temperature (80–130 °C) and catalyzed with potassium acetate, piperidine or diethylamine gave mixtures of mono- and bischalcones with predominant derivative **2a** in 26, 29 and 22% yields, respectively.

As a result of this study, the conditions described in entries 1 (method *B*) and 9 (method *A*) (see Experimental) were used for preparation of compounds **1a–1p** and **2a–2g**.

Compound **1a** was then subjected to catalytic hydrogenation to confirm the influence of 2-oxopropenyl moiety on the antimycobacterial activity of the studied compounds. The reaction was carried out using Pd/C in ethyl acetate and compound **5a** was isolated in an almost quantitative yield (Scheme 2).

Spectral characteristics of all new compounds correspond to the proposed structures. In IR spectra (Table III) of **1** and **2**, characteristic vibrations can be found in the 1 623–1 674 cm^{-1}

TABLE II
The influence of reaction conditions on the yield of derivatives **1a** and **2a** (10 mmol of compound **3** have been used in all experiments)

| Entry	**4a** mmol	Agents	T, °C	Time h	Yield, %	
					1a	**2a**
1	32	2 ml H_2SO_4/40 ml CH_3COOH	25	336	17	58
2	32	1 ml H_2SO_4/40 ml CH_3COOH	25	96	–	–
3	32	3 ml H_2SO_4/40 ml CH_3COOH	25	240	59	12
4	40	1 mmol KOH/1 ml H_2O/12 ml CH_3OH	25	3	70	4
5	40	1 mmol KOH/15 ml CH_3OH	5	8	5	10
6	40	1 mmol KOH/15 ml CH_3OH	60	3	8	3
7	40	1 mmol $Ba(OH)_2$/15 ml CH_3OH	60	3	11	4
8	40	1 mmol $Ba(OH)_2$/15 ml CH_3OH	25	4	72	2
9	40	1 mmol KOH/15 ml CH_3OH	25	5	74	3
10	40	10 mmol KOH/15 ml CH_3OH	25	2	30	8
11	23	30 mmol CH_3COOK/20 ml $(CH_3CO)_2O$	130	2	22	26
12	40	1 mmol piperidine/20 ml CH_3OH	80	28	12	29
13	30	3 mmol diethylamine/6 ml pyridine	100	20	11	22

region proving thus the presence of carbonyl group in the –CH=CH–CO– moiety. In addition, an intensive absorption band exhibiting minimal variability is present in the spectra of **2**, at 1 690 cm^{-1} corresponding to the C=O vibration of acetyl group directly bonded to a pyridine ring.
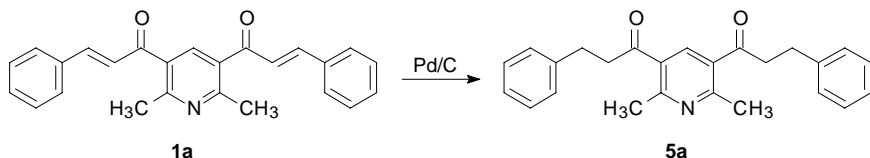
SCHEME 2

The UV-VIS spectra of compounds **1** and **2** are summarized in Table III. The presence of two distinct maxima at 220 and 320 nm indicates typical $\pi \to \pi^*$ transitions of the conjugated system[19], which is corroborated by relatively high values of log ε. In accordance with literature data[20], the substitution of the *para*-position in phenyl ring by electron-donating groups leads to a considerable bathochromic shift; at the same time, the electron-withdrawing substituents cause only minimal hypsochromic changes[21].

The MS spectra of compounds **1** and **2** are very similar (Tables IV and V). Nevertheless, their fragmentation is not fully identical. The characteristic fragmentation mode of these compounds has been already described[22–28] in the basic chalcone series. MS spectra of azachalcones **2** (Scheme 3) consist of molecular ion **a** and additional peak **b** [M – H]$^+$.
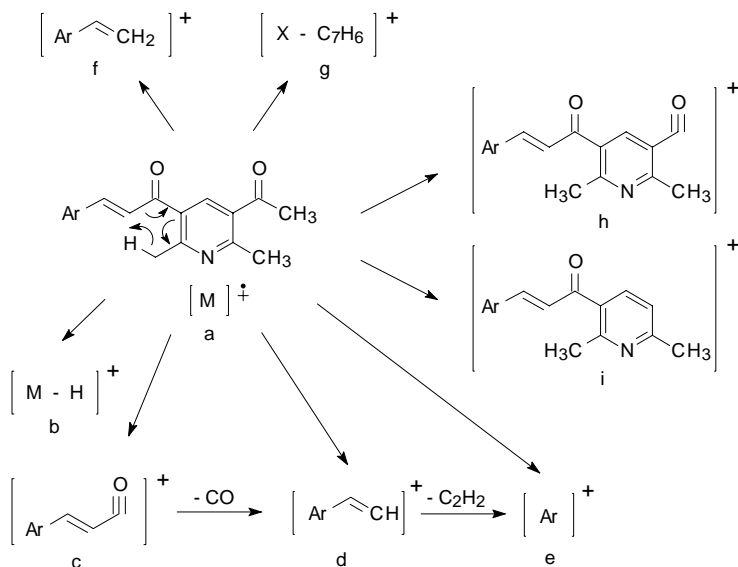
SCHEME 3

TABLE III
Characteristic IR and UV-VIS data of compounds **1**, **2** and **5**

Compound	ν, cm^{-1}	λ_{max}, nm	$\log \varepsilon^{a}$
1a	3 015, 1 642, 1 601, 1 576	225, 304	4.54, 4.70
1b	3 023, 1 652, 1 590, 1 525	258, 414	4.46, 4.87
1c	3 632, 3 412, 3 019, 1 729, 1 688, 1 600, 1 585, 1 514, 1 252	240, 343	4.22, 4.53
1d	3 024, 1 665, 1 639, 1 598	228, 316	4.41, 4.69
1e	3 023, 1 644, 1 600, 1 508, 1 239	222, 307	4.50, 4.63
1f	3 024, 1 644, 1 591, 1 401	224, 311	4.47, 4.70
1g	3 016, 1 645, 1 603, 1 586, 1 488	228, 310	4.45, 4.40
1h	3 023, 1 636, 1 598, 1 513, 1 254	239, 337	4.40, 4.65
1i	3 024, 2 232, 1 671, 1 650, 1 606	221, 295	3.88, 3.91
1j	3 018, 1 673, 1 597, 1 525, 1 347	215, 308	3.71, 3.74
1k	3 025, 1 721, 1 647, 1 605, 1 284	227, 301	4.40, 4.58
1l	3 015, 1 623, 1 599, 1 552	245, 337	4.41, 4.77
1m	3 014, 1 638, 1 608, 1 588	285, 340	4.37, 4.65
1n	3 014, 1 639, 1 617, 1 595	213, 238, 316	4.58, 4.32, 4.66
1o	2 979, 1 689, 1 648, 1 607, 1 588	300	4.74
1p	2 960, 1 672, 1 659, 1 614, 1 585	280	4.68
2a	3 024, 1 690, 1 670, 1 646, 1 597	228, 304	4.34, 4.33
2b	3 011, 1 688, 1 590, 1 526	259, 416	4.07, 4.44
2c	3 587, 3 350, 3 025, 1 691, 1 595, 1 514	235, 342	3.90, 3.96
2d	3 016, 1 689, 1 641, 1 596	233, 318	4.36, 4.47
2e	3 022, 1 690, 1 600, 1 500	229, 308	4.29, 4.37
2f	3 012, 1 690, 1 645, 1 591, 1 492	231, 312	4.35, 4.42
2g	3 016, 1 690, 1 644, 1 590, 1 488	231, 312	4.38, 4.38
5a	3 028, 3 013, 1 688, 1 590	246, 278	4.16, 3.95

[a] ε is given in m^{2}/mol.

TABLE IV
Mass spectra of compounds **2**

Compound	a	b	c	d	e	f	g	h	i	M – e	M – g	M – CO	M – X
						Characteristic ions, *m/z* (relative intenzity, %)							
2a	279 (89)	278 (24)	131 (87)	103 (100)	77 (98)	104 (25)	91 (56)	264 (10)	236 (25)	202 (16)	188 (67)	251 (3)	–
2b	322 (45)	321 (15)	174 (28)	146 (46)	120 (20)	147 (60)	134 (100)	307 (11)	279 (5)	202 (3)	188 (1)	294 (1)	278 (5)
2c	295 (45)	294 (26)	147 (78)	119 (52)	93 (3)	120 (8)	107 (100)	280 (12)	252 (12)	202 (21)	188 (55)	267 (2)	278 (12)
2d	293 (31)	292 (15)	145 (35)	117 (45)	91 (31)	118 (8)	105 (100)	278 (26)	250 (9)	202 (9)	188 (9)	265 (1)	278 (26)
2e	297 (78)	296 (28)	149 (79)	121 (100)	95 (14)	122 (10)	109 (81)	282 (10)	254 (22)	202 (20)	188 (91)	269 (3)	278 (1)
2f	313 (53)	312 (24)	165 (76)	137 (47)	111 (7)	138 (28)	125 (77)	298 (12)	270 (17)	202 (24)	188 (100)	285 (2)	278 (51)
2g	357 (17)	356 (7)	209 (25)	181 (15)	155 (3)	182 (4)	169 (26)	342 (5)	314 (6)	202 (19)	188 (80)	329 (1)	278 (40)

TABLE V
Mass spectra of compounds **1**

Compound	a	b	c	d	e	f	g	M – e	M – g	M – CH₃	M – X	M – CO
1a	367 (22)	366 (10)	131 (81)	103 (100)	77 (67)	104 (12)	91 (45)	290 (7)	276 (17)	351 (1)	–	339 (1)
1b	453 (15)	452 (5)	174 (24)	146 (43)	120 (12)	147 (15)	134 (100)	333 (1)	319 (1)	438 (6)	409 (1)	425 (0)
1c	399 (22)	398 (10)	147 (89)	119 (91)	93 (4)	120 (25)	107 (100)	306 (4)	292 (15)	384 (1)	382 (4)	371 (0)
1d	395 (16)	394 (6)	145 (35)	117 (48)	91 (32)	118 (6)	105 (100)	304 (3)	290 (6)	380 (8)	380 (8)	367 (0)
1e	403 (24)	402 (9)	149 (74)	121 (100)	95 (10)	122 (12)	109 (83)	308 (6)	294 (22)	388 (1)	384 (1)	375 (0)
1f	435 (15)	434 (7)	165 (100)	137 (77)	111 (6)	138 (12)	125 (75)	324 (7)	310 (27)	420 (1)	400 (14)	407 (0)
1g	523 (3)	522 (2)	209 (20)	181 (15)	155 (2)	182 (5)	169 (24)	368 (2)	354 (7)	508 (0.3)	444 (3)	495 (0)
1h	427 (4)	426 (2)	161 (19)	133 (19)	107 (1)	134 (3)	121 (100)	320 (1)	306 (1)	412 (0.5)	396 (2)	399 (0)
1i	417 (10)	416 (6)	156 (60)	128 (100)	102 (39)	129 (12)	116 (11)	315 (7)	301 (19)	402 (0.5)	391 (0.5)	389 (0)
1j	457 (8)	456 (3)	176 (66)	148 (5)	122 (13)	149 (4)	136 (63)	335 (9)	321 (15)	442 (1)	382 (1)	429 (0)
1k	483 (8)	482 (6)	186 (8)	161 (4)	135 (3)	162 (12)	149 (5)	348 (4)	334 (9)	468 (9)	424 (7)	455 (0)
1l	347 (85)	346 (20)	121 (88)	93 (13)	67 (0)	94 (0)	81 (100)	280 (1)	266 (25)	332 (4)	–	319 (13)
1m	379 (55)	378 (25)	137 (80)	109 (62)	83 (2)	110 (6)	97 (100)	296 (6)	282 (21)	364 (4)	–	351 (3)
1n	379 (95)	378 (33)	137 (100)	109 (83)	83 (2)	110 (5)	97 (60)	296 (1)	282 (22)	264 (2)	–	351 (4)
1o	369 (95)	368 (35)	132 (100)	104 (89)	78 (30)	105 (15)	93 (51)	291 (27)	277 (19)	354 (4)	–	341 (64)
1p	369 (100)	368 (32)	132 (98)	104 (60)	78 (45)	105 (11)	93 (71)	291 (55)	277 (21)	354 (3)	–	341 (33)

Characteristic ions, m/z (relative intenzity, %)

During the following fragmentation of **a**, the major part of ions is created by the direct bond cleavage, the most important fragmentation mode being the cleavage of the bond adjacent to carbonyl group giving ions **c** and **d**. Another possible explanation of the presence of ion **d** could be elimination of CO from fragment **c**. The presence of ion **e** can be explained by elimination of ethyne molecule from ion **d**, although the presence of [M – **e**]$^+$ ion does not exclude the possibility of its direct formation. Ion **f**, possessing low relative intensity, is formed by hydrogen transfer from methyl group in position 2 of the pyridine ring. This so called ortho effect[24] proceeds *via* a six-membered cyclic mechanism in which hydrogen is transferred to a double bond of the chalcone moiety. Easy and unexpected formation of ion **c** (25–87% rel. int.) could be rationalized by the breaking of the bond between the 2-oxopropenyl and pyridine moieties. The less intensive **h** and **i** ions have their origin in elimination of methyl or acetyl radical from molecular ion **a**, which proves the presence of an acetyl group in compounds **2**. The very intensive fragmentation mode (26–100% rel. int.) is characterized by the formation of substituted tropylium ion **g** in the spectra of azamonochalcones **2a–2b**. Elimination of carbon monoxide from molecular ion (Table IV), which was observed in simple chalcone derivatives[25,28], is not so intensive in this series (1–3% rel. int.).

The fragmentation mechanism in symmetrical bis substituted compounds **1** is clearly simpler (Scheme 4) with a fragmentation pattern typical of chalcones, *i.e.*, cleavage of α bonds linked to carbonyl group. The most intensive mode is again the formation of a substituted tropylium cation **g** or its heteroanalogs, such as pyrylium ($C_5H_5O^+$) (**1l**), thiapyrylium ($C_5H_5S^+$) (**1m**, **1n**), and azepinium ($C_6H_5N^+$) (**1o**, **1p**). The formation of intensive ions **c**, **d**, **e**, and **f** from molecular ion **a** is the same as described above for
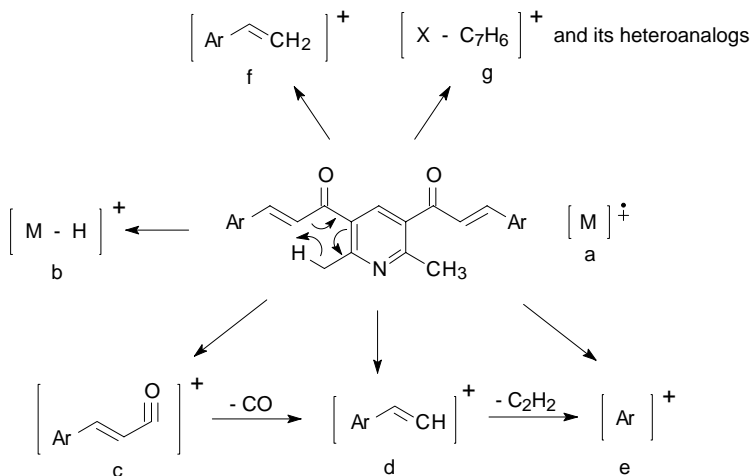
SCHEME 4

^{1}H NMR data (δ, ppm; J, Hz) of compounds **1**, **2** and **5**

Compound	Pyridine		Benzene	CH=CH (d)	Other
	CH$_3$ (s)	H-4 (s)			
1a	2.71	7.90	7.39–7.44 m, 6 H; 7.56–7.59 m, 4 H	7.13; 7.51 J = 16.06	–
1b	2.67	7.80	6.66 d, 4 H; 7.45 d, 4 H	6.89; 7.40 J = 15.85	3.04^a
1c	2.69	7.84	6.86 d, 4 H; 7.48 d, 4 H	6.98; 7.43 J = 16.50	3.54^b
1d	2.70	7.88	7.21 d, 4 H; 7.47 d, 4 H	7.08; 7.48 J = 16.07	2.39^c
1e	2.71	7.88	7.09–7.13 m, 4 H; 7.55 m, 4 H	7.05; 7.48 J = 16.04	–
1f	2.71	7.89	7.38 d, 4 H; 7.51 d, 4 H	7.09; 7.47 J = 16.08	–
1g	2.71	7.89	7.43 d, 4 H; 7.55 d, 4 H	7.11; 7.45 J = 16.04	–
1h	2.69	7.85	6.92 d, 4 H; 7.52 d, 4 H	6.99; 7.45 J = 16.01	3.85^d
1i	2.73	7.95	7.68 d, 4 H; 7.72 d, 4 H	7.22; 7.54 J = 16.06	–
1j	2.75	7.96	7.74 d, 4 H; 8.28 d, 4 H	7.25; 7.59 J = 16.02	–
1k	2.73	7.94	7.64 d, 4 H; 8.07 d, 4 H	7.20; 7.55 J = 16.08	3.94^e
1l	2.71	7.92	6.53 dd, 2 H; 6.74 d, 2 H; 7.55 d, 2 H	7.03; 7.32 J = 15.65	–
1m	2.70	7.86	7.10 dd, 2 H; 7.34 d, 2 H; 7.47 d, 2 H	6.91; 7.64 J = 15.66	–
1n	2.69	7.83	7.35–7.39 m, 4 H; 7.58 d, 2 H	6.92; 7.47 J = 15.94	–
1o	2.73	7.94	7.36 dd, 2 H; 7.90 ddd, 2 H; 8.65 dd, 2 H; 8.79 d, 2 H	7.21; 7.54 J = 16.08	–
1p	2.74	7.95	7.42 dd, 4 H; 8.70 dd, 4 H	7.28; 7.47 J = 16.06	–
2a	2.61 2.68	8.07	7.42–7.46 m, 3 H; 7.58–7.60 m, 2 H	7.13; 7.50 J = 16.09	– 2.81^f
2b	2.60 2.64	8.01	6.67 d, 2 H; 7.46 d, 2 H	6.89; 7.38 J = 15.85	3.06^a 2.80^f
2c	2.62 2.65	8.05	6.88 d, 2 H; 7.48 d, 2 H	6.97; 7.41 J = 16.00	6.60^b 2.81^f

TABLE VI
(*Continued*)

Compound	Pyridine		Benzene	CH=CH (d)	Other
	CH$_3$ (s)	H-4 (s)			
2d	2.61 2.66	8.05	7.24 d, 2 H; 7.48 d, 2 H	7.08; 7.46 $J = 16.04$	2.40[c] 2.80[f]
2e	2.62 2.67	8.05	7.10–7.15 m, 2 H; 7.57–7.60 m, 2 H	7.05; 7.47 $J = 16.06$	– 2.80[f]
2f	2.62 2.67	8.06	7.41 d, 2 H; 7.52 d, 2 H	7.10; 7.46 $J = 16.04$	– 2.80[f]
2g	2.62 2.67	8.06	7.45 d, 2 H; 7.57 d, 2 H	7.11; 7.45 $J = 16.03$	– 2.80[f]
5a	2.68	7.85	7.17–7.22 m, 6 H; 7.26–7.30 m, 4 H	–	3.02–3.06 m, 4 H[g]; 3.12–3.16 m, 4 H[g]

[a] –(CH$_3$)$_2$N–; [b] –HO–; [c] –CH$_3$–; [d] H$_3$O–; [e] –CH$_3$OOC–; [f] CH$_3$CO–; [g] CH$_2$–CH$_2$–.

azachalcones **2**. A molecular rearrangement[25,28] accompanied by extrusion of CO is observable in derivatives **1l–1p**, whereas compounds **1b–1k** do not show this behaviour under the measuring conditions.

The MS spectrum of saturated derivative **5a** corresponds with the well-known fragmentation of carbonyl compounds (see Experimental).

^{1}H NMR spectra of symmetrical compounds **1b–1k** (Table VI) exhibit typical AA′XX′ or AA′BB′ splitting pattern for *para*-substituted phenyl rings in the region 6.66–8.28 ppm depending on the substitution. All derivatives **1a–1p** show a signal of position 4 of the pyridine ring, which can be found as a singlet at 7.83–7.96 ppm. The doublets of the CH=CH–CO moiety are situated in the 6.89–7.64 ppm region with typical coupling constants 15.6–16.1 Hz proving *trans*-configuration of the double bond. Methyl groups in 2 and 6 positions of the pyridine ring give a singlet at 2.7 ppm. In addition, the ^{1}H NMR spectra of non-symmetrical azachalcones **2** also contain a singlet at about 2.80 ppm due to the acetyl group.

Preliminary Results of Biological Activity Studies

Substituted chalcones exhibit a distinct antimycobacterial *in vitro* efficiency measured on a suitable cultivating medium by the dilution method[29]. Activity of compound **1a** against *Mycobacterium tuberculosis* H$_{37}$Rv (minimum inhibition concentration (MIC)

0.25 µg/ml), *M. kansassi* PKG$_8$ (MIC 5 µg/ml), and *M. avium* 80/72 (MIC 50 µg/ml) is comparable with that of isoniazide (INH), and is better than activity of ethionamide (2-ethylisonicotinthioamide), PAS (4-amino-2-hydroxybenzoic acid) or conteben (4-acetamidobenzaldehyde thiosemicarbazone). *In vivo* experiments in mice showed acute toxicity (dosis tolerata maxima) of approximately 2 000 mg/kg while the published value for INH is 125 mg/kg (ref.[30]). A detailed study of the antimycobacterial activity of all compounds **1** and **2** has been published separately[31].

EXPERIMENTAL

Temperature data were not corrected. The melting points were determined on a Boetius block (Zeiss, Jena, Germany). The IR spectra were measured on an FT-IR spectrometer Nicolet 740 in chloroform. ^{1}H NMR spectra were obtained with a Bruker 400 spectrometer in deuteriochloroform; chemical shifts are reported in δ (ppm) with TMS as an internal standard, the coupling constants *J* are given in Hz. Mass spectra were measured on a Varian MAT 44S instrument with ionization energy 70 eV. The UV-VIS spectra were collected on an Otsuka MCPD 1100 spectrophotometer in ethanol. The purity of the substances and the course of reactions were monitored by TLC using Silufol and Alufol plates (Kavalier, Votice, Czech Republic). Column preparative chromatography was carried out on silica gel Merck (40/100).

3,5-Diacetyl-2,6-dimethyl-1,4-dihydropyridine

A mixture of aqueous formaldehyde (36%; 13.6 ml, 0.4 mol), pentane-2,4-dione (26.2 g, 0.29 mol), and aqueous solution of ammonium carbonate (10%; 600 ml, 0.62 mol) was allowed to stay for 48 h at room temperature with intermittent stirring. The yellow precipitate was collected by suction and dried at room temperature to yield 26.7 g (95%) of yellow crystals with m.p. 195–208 °C. The pure product was obtained by crystallization from aqueous ethanol, m.p. 213–215 °C (ref.[32] gives m.p. 207–208 °C).

3,5-Diacetyl-2,6-dimethylpyridine (3)

Aqueous H_2SO_4 (20%, 199 ml) was added dropwise during 2 h to an ice cool (0–5 °C), vigorously stirred mixture of 3,5-diacetyl-2,6-dimethyl-1,4-dihydropyridine (30.6 g, 0.16 mol), diethyl ether (800 ml), sodium nitrite (61.2 g, 0.89 mol) and water (300 ml). The reaction mixture was stirred for additional 6 h at the same temperature and then for 15 h at room temperature. The content was alkalinized with 20% aqueous NaOH solution (220 ml) and the mixture was extracted with diethyl ether (4 × 200 ml). The combined organic layers were washed with brine (100 ml), dried over potassium carbonate and evaporated to dryness. The crude product (15.8 g, 52%) was crystallized from cyclohexane to yield **3** (13.8 g, 46%), m.p. 66–69 °C (ref.[32] gives m.p. 69–70 °C (petroleum ether)).

1,1′-(2,6-Dimethyl-3,5-pyridinediyl)bis(3-phenyl-(*E*)-prop-2-en-1-one) (**1a**) and 1-(5-Acetyl-2,6-dimethylpyridin-3-yl)-3-phenyl-(*E*)-prop-2-en-1-one (**2a**)

Method A. To an ice cool solution of 3,5-diacetyl-2,6-dimethylpyridine (**3**) (1.92 g, 10.0 mmol) in methanol (40 ml), benzaldehyde (**4a**) (4.24 g, 40 mmol) and powdered potassium hydroxide (50 mg, 0.89 mmol) were gradually added. The reaction mixture was stirred for 30 min at 5 °C and then for 5 h at room temperature. The mixture was diluted with 15 ml of water and extracted with dichloromethane (3 × 30 ml). The combined organic layers were washed with water (20 ml), brine (20 ml)

and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by column chromatography on silica gel (350 g SiO_2, hexane–*t*-butyl methyl ether). The corresponding fractions were collected, evaporated and crystallized from methanol to yield **1a** (2.7 g, 74%) as yellowish crystals, m.p. 88–90 °C, and **2a** (56 mg, 2%), m.p. 78–84 °C.

Method B. To a solution of 3,5-diacetyl-2,6-dimethylpyridine (**3**) (1.92 g, 10 mmol) and benzaldehyde (**4a**) (3.2 ml, 32 mmol) in glacial acetic acid (40 ml), concentrated H_2SO_4 (2 ml, 38 mmol) was added and the mixture was stirred for 14 days at room temperature. The reaction mixture was then diluted with water (200 ml) and extracted with dichloromethane (3 × 40 ml). The combined extracts were washed consecutively with water (30 ml), a saturated aqueous solution of $Na_2S_2O_5$ (30 ml), and brine (30 ml). The product was then isolated in a similar manner as described above in method *A* to yield azachalcone **1a** (0.63 g, 17%), m.p. 88–90 °C, and **2a** (1.62 g, 58%), m.p. 78–84 °C.

1,1′-(2,6-Dimethyl-3,5-pyridinediyl)bis(3-phenylpropan-1-one) (**5a**)

A mixture of compound **1a** (1.0 g, 2.7 mmol), 10% Pd/C (100 mg), and ethyl acetate (25 ml) was hydrogenated at room temperature under a common pressure. The reaction was stopped after consumption of 2 equivalents of hydrogen, the catalyst was filtered off and the solvent was evaporated. The residue (1.02 g) was purified by column chromatography on silica gel (35 g SiO_2, hexane–*t*-butyl methyl ether 3 : 1) and subsequently by crystallization from an ethyl acetate–hexane mixture to yield **5a** (877 mg, 87%) as white crystals, m.p. 85–87 °C. MS spectrum, *m/z* (rel.%): $[M]^{+\bullet}$ = 371 (34 rel.%); $[M – C_6H_6]^+$ = 293 (2.5); $[M – (Ph–CH_2)]^+$ = 280 (2.6); $[M – (Ph–CH_2CH_2)]^+$ = 266 (100); $[M – (Ph–CH_2CH_2CO)]^+$ = 238 (16); $[Ph–CH_2CH_2CO]^+$ = 133 (8); $[Ph–CH_2CH_2]^+$ = 105 (43); $[Ph–CH_2]^+$ = 91 (65); $[Ph]^+$ = 71 (26).

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