1,3-Dipolar cycloadditions of heterocycles XXII.* Synthesis and biological activity of condensed isoxazolines

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The synthesis of condensed isoxazolines, containing an *N*-arylmaleinimide and an isoxazoline skeleton in the molecule is described. Thus the substituted 3,5-diaryl-4,6-dioxo-3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]-isoxazoles were synthesized by a 1,3-dipolar cycloaddition of substituted benzonitrile oxides to substituted phenylmaleinimides, in which the substituent was H, Cl, F, Br, NO_2, OCH_3, CH_3, $CH(CH_3)_2$, CF_3, respectively. The prepared condensed isoxazolines were active against yeasts, moulds, and bacteria.

Within the scope of our pesticide program papers on the fungicidal activity of arylmaleinimide derivatives caught our attention. Thus compounds with a dicarboximide type structure were found to possess systemic activity against *Botrytis cinerea*, *Cochliobolus miyabeanus*, and *Pellicularia sasaki* [1]. The parent derivative, *N*-(3,5-dichlorophenyl)pyrrolo-2,5-dione (*I*, Dimetachlon) has been applied as a protective and currative fungicide [2]. Recently, a whole series of excellent fungicides was discovered, in which the heterocyclic part of the structure was either a pyrrolidinedione, an oxazolidinedione or an imidazoledione [3, 4]. Moreover, the fungicidal and herbicidal properties of isoxazoline derivatives have been well known [1, 5]. We therefore set out to prepare condensed isoxazolines, containing a pyrrolidinedione skeleton, with the aim to test their activity against bacteria, yeasts, and moulds.

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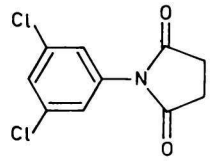

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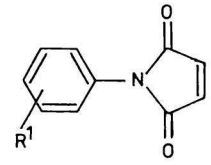

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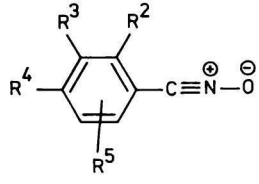

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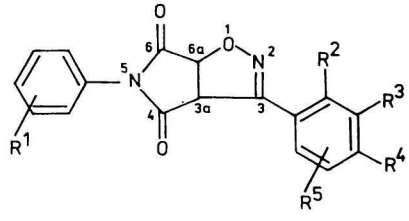

IV-1—IV-56

The title condensed isoxazolines *IV* (*IV-1* through *IV-56*) were prepared by a 1,3-dipolar cycloaddition of substituted benzonitrile oxides *III*, in which R^2—R^5 was H, 4-CH_3, 4-F, 3-F, 2-F, 4-Cl, 3-Cl, 2-Cl, 2,4-diCl, 3,4-diCl, 4-NO_2, 3-NO_2, 4-Br, 3-Br, 2-Br, 4-OCH_3, and 2-OCH_3, respectively, to *N*-aryl-substituted maleinimides *II*, where R^1 stands for 4-F, 3-F, 2-F, 4-Cl, 4-Br, 4-$CH(CH_3)_2$, 3-CF_3, and 3,4-diCl. Substituted benzonitrile oxides *III* we obtained by releasing them *in situ* from the corresponding *N*-hydroxybenzenecarboximidoyl chlorides by the action of triethylamine in ether in the presence of dipolarophile *II* at 0 °C [6]. The alternative method [7], which utilizes the oximes and sodium hypochlorite failed in our conditions, since it led to ring-opening of pyrrolidinedione, as reported earlier [8]. The 1,3-dipolar cycloaddition gave fair to excellent yields (32—97 %). The structure assignment of derivatives *IV* was based on the analysis of spectral data (Tables 1 and 2). Thus, for example, ^{1}H NMR spectrum of 3-phenyl-5-(4-fluorophenyl)-4,6-dioxo-3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole (*IV-1*) displays two typical doublets of the bridgehead protons at $\delta = 5.10$ (H-3a) and $\delta = 5.76$ (H-6a), with a coupling constant $^3J_{3a,6a} = 10$ Hz, testifying to the presence of a *cis* arrangement of the hydrogen atoms. The ^{13}C NMR signals of bridgehead carbon atoms were observed at $\delta = 56.18$ (C-3a) and $\delta = 82.39$ (C-6a). The singlets at $\delta = 154.00$, 171.60, and 172.50 were assigned to C-3, C-4, and C-6 atoms, respectively. ^{1}H and ^{13}C chemical shifts of compounds *IV-2*—*IV-56* fell within the range: 4.39—5.59 (H-3a), 5.63—5.99 (H-6a), 54.12—58.58 (C-3a), and 80.04—83.44 (C-6a). All the compounds *IV* also show the typical UV spectra of condensed isoxazolines [9].

Table 1

Characterization of the prepared compounds *IV-1—IV-56*

Compound *IV*	R^1	R^2	R^3	R^4	R^5	Yield %	M. p. °C	^{1}H NMR/δ H-3a	H-6a	H_{arom}	UV spectrum λ_{max} (log ε)
1[a]	4-F	H	H	H	H	91	183—184	5.10	5.76	7.07—7.46, 7.92—8.05	257 (2.41)
2	4-F	H	H	Cl	H	70	192—194	5.31	5.78	7.20—7.52, 8.00	265.9 (2.62)
3	4-F	Cl	H	Cl	H	93	192—194	5.43	5.87	7.25—7.86	244 (2.58)
4	4-F	H	NO_2	H	H	89	219—221	5.50	5.77	7.22—8.86	254 (2.69)
5[b]	4-F	H	H	NO_2	H	88	229—231	5.48	5.85	7.31—7.37, 8.14—8.39	238 (2.52), 298 (2.51)
6	4-F	H	H	F	H	51	204—206	5.37	5.82	7.05—7.49	254 (2.53)
7	4-F	Cl	H	H	H	85	167—169	5.33	5.76	7.13—7.70	242 (2.38)
8	4-F	H	Cl	Cl	H	43	182—184	5.48	5.83	7.22—8.17	268 (2.61)
9	4-F	H	Cl	H	H	49	131—133	5.35	5.81	7.20—8.02	257 (2.51)
10	4-F	Cl	H	H	6-Cl	87	213—214	5.08	5.97	7.25—7.58	242 (2.26)
11[a]	4-F	F	H	H	H	88	168—170	5.20	5.67	7.04—7.84	250 (2.56)
12[a]	4-F	H	F	H	H	79	161—163	4.94	5.70	7.14—7.85	255.5 (2.55)
13[c]	4-F	H	H	OCH_3	H	64	210—211	5.32	5.78	7.01—8.04	276.2 (2.43)
14[d]	4-F	H	OCH_3	H	H	52	139—140	5.35	5.82	7.07—7.64	258 (2.43)
15[a]	4-Cl	H	H	H	H	49	191—193	4.93	5.63	7.12—7.47, 7.92—8.03	256.7 (2.69)
16[b]	4-Cl	H	H	Cl	H	95	211—212	5.38	5.76	7.23—7.92	267 (2.30)
17[a]	4-Cl	Cl	H	Cl	H	89	183—184	5.37	5.81	7.17—7.60	251.6 (2.66)
18[b]	4-Cl	H	NO_2	H	H	73	218—220	5.62	5.96	7.38—7.98, 8.45, 8.85	255.2 (2.83)
19[b]	4-Cl	H	H	NO_2	H	94	227—229	5.45	5.81	7.22—7.56, 8.06—8.35	249 (2.38), 297 (2.30)
20	4-Cl	H	H	F	H	40	212—214	5.39	5.82	7.07—7.60	255 (2.73)
21[b,e]	4-Cl	H	H	CH_3	H	80	219—221	5.32	5.70	7.20—7.95	258 (2.49)

Table 1 (Continued)

Compound *IV*	R^1	R^2	R^3	R^4	R^5	Yield	M. p.	^{1}H NMR/δ			UV spectrum
						%	°C	H-3a	H-6a	H_{arom}	λ_{max} (log ε)
22[b]	4-Cl	Cl	H	H	H	87	193—194	5.42	5.93	7.32—7.90	250 (2.40)
23	4-Cl	H	Cl	Cl	H	32	165—167	5.37	5.85	7.28—8.20	262 (2.67)
24[a]	4-Cl	H	Cl	H	H	58	143—146	5.13	5.83	7.28—7.45, 7.98	256.7 (2.40)
25[f]	4-Cl	H	H	OCH_3	H	58	196—198	5.32	5.77	7.00—8.03	271.7 (2.66)
26[g]	4-Cl	H	OCH_3	H	H	67	149—152	5.37	5.83	7.03—7.67	255.4 (2.60)
27	4-Br	H	H	Cl	H	86	220—222	5.40	5.85	7.29—7.98	259 (2.57)
28	4-Br	H	NO_2	H	H	77	205—208	5.52	5.91	7.30—7.85	254 (2.75)
29	4-Br	H	H	F	H	54	215—217	5.36	5.70	7.22—8.12	256 (2.60)
30[a]	4-Br	Cl	H	H	H	91	199—201	5.36	5.77	7.10—7.95	252 (2.51)
31	4-Br	H	H	Br	H	91	231—233	5.37	5.82	7.30—7.98	261 (2.64)
32	4-Br	Br	H	H	H	93	202—204	5.39	5.86	7.30—7.85	252 (2.39)
33[h]	4-Br	H	H	OCH_3	H	97	203—205	5.33	5.78	7.01—8.04	265.1 (2.59)
34[i]	4-Br	H	OCH_3	H	H	52	160—161	5.37	5.84	7.05—7.74	256.9 (2.64)
35[j]	4-$CH(CH_3)_2$	H	H	Cl	H	62	211—213	5.37	5.83	7.19—8.11	264 (2.59)
36[k]	4-$CH(CH_3)_2$	H	NO_2	H	H	84	198—201	5.52	5.92	7.21—8.91	254 (2.74)
37[l]	4-$CH(CH_3)_2$	Cl	H	H	H	62	170—173	5.43	5.88	7.19—7.79	245.5 (2.48)
38[a]	2-F	H	H	Cl	H	66	173—175	4.99	5.74	7.26—8.09	266.5 (2.48)
39[a]	2-F	Br	H	H	H	61	112—115	5.36	5.77	7.19—7.62	
40[a]	2-F	H	H	F	H	67	172—174	5.00	5.73	7.05—8.10	256 (2.48)
41[a]	2-F	F	H	H	H	42	107—110	5.25	5.75	7.08—7.89	249 (2.64)
42[a]	2-F	H	F	H	H	70	114—117	5.03	5.77	7.19—7.84	253 (2.50)
43	2-CF_3	H	H	Cl	H	63	188—189	5.42	5.88	7.49—8.11	267 (2.50)
44	3-CF_3	H	NO_2	H	H	61	162—165	5.59	5.99	7.65—8.91	254.3 (2.40)
45	2-CF_3	H	Br	H	H	77	184—186	5.44	5.89	7.37—8.22	262 (2.30)
46[m]	2-CF_3	OCH_3	Cl	H	5-Cl	84	155—157	5.49	5.89	7.61—7.87	
47[a]	3-F	H	H	Cl	H	87	175—178	5.00	5.72	7.02—7.98	258.7 (2.38)
48[a]	3-F	H	H	F	H	70	168—170	5.06	5.77	7.07—8.13	249 (2.45)
49[a]	3-F	H	H	Br	H	97	155—157	5.04	5.76	7.03—7.46	266.4 (2.52)

Table 1 (Continued)

Compound *IV*	R^1	R^2	R^3	R^4	R^5	Yield	M. p.	^{1}H NMR/δ			UV spectrum
						%	°C	H-3a	H-6a	H_{arom}	λ_{max} (log ε)
50[a]	3-F	F	H	H	H	94	102—105	5.27	5.76	7.01—7.90	247 (2.41)
51[a]	3-F	H	F	H	H	54	129—132	4.85	5.70	6.92—7.81	250.9 (2.70)
52	3-CF_3	H	NO_2	H	H	74	185—188	5.42	5.88	7.49—8.11	250.9 (2.69)
53	3-CF_3	Cl	H	H	H	55	133—136	5.51	5.93	7.44—7.82	245 (2.43)
54	3-CF_3	Br	H	H	H	75	148—150	5.48	5.94	7.40—7.84	244 (2.60)
55[a]	3,4-diCl	H	H	Cl	H	83	195—196	5.22	5.75	7.24—7.95	260.7 (2.66)
56[a]	3,4-diCl	Cl	H	H	H	81	190—191	5.38	5.74	7.24—7.46	

^{1}H NMR spectrum: *a*) $CDCl_3$, *b*) DMSO, *c*) 3.88 (s, 3H, OCH_3), *d*) 3.86 (s, 3H, OCH_3), *e*) 2.46 (s, 3H, CH_3), *f*) 3.87 (s, 3H, OCH_3), *g*) 3.86 (s, 3H, OCH_3), *h*) 3.89 (s, 3H, OCH_3), *i*) 3.87 (s, 3H, OCH_3), *j*) 1.21 (d, 6H, $2\times CH_3$) and 3.02—3.23 (m, 1H, CH), *k*) 1.25 (d, 6H, $2\times CH_3$) and 3.05—3.18 (m, 1H, CH), *l*) 1.25 (d, 6H, $2\times CH_3$) and 2.94—3.17 (m, 1H, CH), *m*) 3.99 (s, 3H, OCH_3).

Table 2

^{13}C chemical shifts (δ) of the prepared compounds *IV-1—IV-56*

Compound *IV*	C-3a	C-6a	C=N*	C=O*	C_{arom}		
1[b]	56.18	82.39	154.00	171.60, 172.50	131.53, 129.43, 116.15	130.01, 128.78,	129.72, 117.08,
2	55.95	82.51	153.18	171.49, 172.31	136.97, 129.95,	135.16, 129.60,	130.30, 128.96
3	57.88	81.98			137.30, 128.49,	130.89, 117.14,	129.89, 116.20
4[b]	54.94	81.85	152.29	170.89, 171.83	147.84, 129.12, 116.43,	133.92, 125.26, 115.49	130.41, 122.57,
5[b]	54.88	82.09	152.46	170.78, 171.60	148.43, 123.80,	133.33, 116.43,	129.47, 115.49
6	55.46	81.73	152.40	170.94, 171.70	134.56, 128.94,	130.64, 128.59,	129.35, 124.32
7	58.17	81.75	153.41	172.66, 170.79	133.64, 131.12, 128.14,	132.58, 129.83, 127.49,	132.35, 129.48, 117.14
8	55.83	82.86	152.53	171.55, 172.25	135.28, 131.71, 128.43,	134.92, 130.60, 123.22	133.11, 129.66,
9	55.95	82.68	153.18	172.37	135.28, 131.24, 129.72, 117.14	134.92, 130.65, 128.55,	131.36, 130.07, 127.26,
10	58.29	81.86	151.21	170.44, 172.72	135.69, 129.43,	133.58, 117.37,	129.78, 116.44
11[a]	55.78	80.04	155.37	169.36, 170.81	133.22, 128.19, 124.60,	132.87, 127.84, 117.08,	130.14, 124.76, 116.69
12[a]	54.12	80.68	151.29	169.60, 170.42	129.71, 128.54, 126.84, 122.92	129.41, 127.89, 126.72,	128.89, 127.54, 123.04,
13[c]	56.47	82.10	153.53	171.28, 172.60	130.54, 117.14,	130.07, 116.15,	120.94, 114.92
14[d]	55.47	81.68	153.22	170.90, 171.75	131.22, 120.46, 116.66,	129.82, 117.42, 116.13,	129.04, 116.99, 115.82
15[a]	54.94	80.33	152.70	169.95, 170.64	134.75, 128.89, 126.67	131.29, 128.07,	129.47, 127.37,
16[a]	54.87	80.67	151.93	169.60, 170.54	137.48, 129.47, 125.20	135.14, 129.29,	130.11, 127.42,

Table 2 (Continued)

Compound *IV*	C-3a	C-6a	C=N*	C=O*	C_{arom}		
17[a]	56.16	80.38	151.87	169.07, 170.43	132.16, 137.60, 127.32,	134.03, 129.23, 124.32	135.02, 129.47,
18[b]	55.00	81.85	152.33	170.60, 171.59	147.78, 129.00,	133.86, 125.20,	130.41, 122.51
19[b]	54.88	82.09	152.41	170.54, 171.48	148.37, 129.06,	133.33, 128.83,	130.41, 123.74
20	55.52	81.73	152.34	170.77, 171.59	136.70, 130.28,	134.03, 129.17,	130.64, 128.59
21[e]	55.05	80.26	152.63	169.66, 170.77	141.75, 127.94,	134.91, 127.36,	129.52, 123.73
22[b]	56.40	80.15	152.58	169.08, 170.00	131.81, 129.30,	131.17, 127.19,	130.53, 125.61
23[a]	54.53	80.68	151.00	169.26, 170.08	135.68, 130.88, 129.06,	135.27, 129.65, 127.25,	133.39, 129.53, 127.02
24[a]	54.82	80.91	151.76	169.55, 170.54	134.68, 129.24, 127.37,	130.99, 128.30, 126.08	129.94, 127.72,
25[f]	56.47	82.10	153.47	171.61, 172.49	134.75, 129.90, 114.92	131.83, 129.31,	130.54, 120.89,
26[g]	56.30	82.45	154.00	171.49, 172.37	134.87, 130.60, 126.50,	132.76, 129.96, 121.24	131.77, 127.15,
27	56.12	82.59	153.21	171.36, 172.18	136.99, 132.11, 129.82, 122.83	132.89, 130.48, 129.55,	132.80, 130.39, 127.37,
28	55.89	82.97	152.83	171.31, 172.02	149.02, 129.54,	132.88, 125.86,	131.00, 123.40
29	56.24	82.42	153.12	171.43, 172.26	132.92, 131.26, 125.00,	132.82, 131.10, 122.84,	132.16, 129.57, 116.62
30	58.23	81.86	153.35	170.69, 172.42	133.64, 132.00, 128.14,	132.94, 131.12, 127.55,	132.58, 129.43, 122.89
31	56.00	82.56		171.31, 172.13	132.88, 129.54, 122.87	132.58, 127.79,	130.54, 125.33,
32	58.58	81.80	154.29	170.49, 172.48	134.22, 132.41, 122.81	132.94, 129.40,	132.70, 128.66,
33[h]	56.47	82.16	153.53	171.61, 172.48	132.94, 122.82, 116.15,	130.54, 120.94, 114.92	129.60, 117.14,

Table 2 (Continued)

Compound *IV*	C-3a	C-6a	C=N*	C=O*	C_{arom}		
34[i]	56.24	82.39			132.88, 121.18,	130.54, 117.44,	129.55, 117.04
35[j]	56.00	82.62	153.35	171.72, 172.48	136.97, 127.73,	130.42, 127.49	129.60,
36[k]	55.89	83.03	153.06		134.52, 127.73, 123.46	131.00, 127.49,	130.48, 125.86,
37[l]	58.23	81.86	153.53	170.96, 172.83	132.58, 128.14,	132.53, 127.73,	131.06, 127.44
38[a]	55.06	80.80	151.80		131.70, 124.68,	131.40, 117.05,	129.06, 116.31
39[a]	56.75	80.26	151.90	168.31, 170.30	133.62, 127.77, 116.42	131.98, 124.90,	128.88, 117.18,
40[a]	55.23	80.68	151.70	169.37, 169.96	131.75, 130.00, 122.74,	131.40, 128.89, 117.13,	130.35, 124.67, 116.48
41[a]	55.98	80.26	151.93	168.61, 170.01	133.15, 131.34, 124.61,	132.80, 130.11, 117.00,	131.63, 128.14, 116.30
42	55.00	80.86	151.94		131.70, 128.83, 116.31	131.40, 124.68,	130.59, 118.59,
43[m]	56.82	83.03	153.12	171.37, 172.02	137.09, 131.82, 130.42,	134.57, 131.59, 129.66,	132.06, 131.36, 128.08
44[n]	56.71	83.44	152.77	171.84, 171.37	134.63, 131.77, 130.13, 125.97	134.34, 131.41, 128.31,	132.06, 131.06, 127.96,
45[o]	56.76	83.15	153.07	171.00, 171.96	134.57, 131.48, 122.95	134.40, 127.96,	131.88, 127.49,
46[p]	57.76	82.68	151.60	170.90, 172.02	134.63, 131.41, 125.45	132.99, 129.83,	131.82, 128.20,
47[a]	54.93	80.79		169.59, 170.53	139.55, 130.57, 125.13,	137.42, 129.29, 121.47	132.27, 129.17,
48[a]	55.00	80.50		169.41, 170.10	130.41, 115.67,	121.84, 114.32	116.54,
49[a]	55.11	80.67		169.50, 170.59	130.58, 122.97, 115.66,	130.40, 121.80, 114.31	130.11, 116.54,
50[a]	54.58	80.15		169.20, 170.70	132.57, 121.69,	129.94, 115.96	124.38,

Table 2 (Continued)

Compound *IV*	C-3a	C-6a	C=N*	C=O*	C_{arom}		
51[a]	54.71	80.62	151.82	169.32, 170.31	130.59, 121.81, 117.83	130.23, 121.69,	123.80, 118.65,
52[r]	55.95	83.03	152.03	171.36, 172.07	149.26, 131.65, 126.38,	145.57, 131.00, 125.92,	134.52, 130.42, 134.60
53[s]	58.23	81.92	153.29		133.70, 131.12, 126.32,	132.53, 131.00, 124.45	131.47, 128.14,
54	58.58	81.86			134.28, 128.66, 122.81	132.70, 126.32,	131.00, 124.45,
55	55.28	81.20			134.43, 128.23,	129.34, 125.66	129.11,
56	56.39	80.15			133.27, 130.28,	131.99, 127.30,	131.30, 125.70

* With some compounds the signals of carbons C=O and C=N were not observed since these compounds were badly soluble and would have required a very long time of accumulation.

[a] ^{13}C NMR spectrum: *a*) $CDCl_3$, *b*) DMSO, *c*) 55.38 (q, OCH_3), *d*) 54.94 (q, OCH_3), *e*) 21.52 (q, CH_3), *f*) 55.83 (q, OCH_3), *g*) 55.77 (q, OCH_3), *h*) 55.83 (q, OCH_3), *i*) 55.71 (q, OCH_3), *j*) 34.56 (d, CH) and 34.06 (q, $2 \times CH_3$), *k*) 34.53 (d, CH) and 24.06 (q, $2 \times CH_3$), *l*) 34.48 (d, CH) and 24.06 (q, $2 \times CH_3$), *m*) 46.41 (s, CF_3), *n*) 46.41 (s, CF_3), *o*) 46.35 (s, CF_3), *p*) 46.29 (s, CF_3) and 57.35 (q, OCH_3), *r*) 46.18 (s, CF_3), *s*) 46.23 (s, CF_3).

It has been found that the condensed isoxazolines *IV* were effective mainly against yeasts and this activity was therefore studied in more detail. The ED_{50} values are given in Table 3. The inhibitory effect on the growth of *Saccharomyces cerevisiae* was checked against the activity of a standard — potassium sorbate. The results document that some of the studied condensed isoxazolines were better growth inhibitors than the sorbate.

All compounds from the series *IV* were tested against phytopathogenic fungi. Active against *Phytophthora infestans* were the compounds *9, 32, 36*, against *Alternaria species 2, 8, 9, 21, 22, 42*, against *Botrytis cinerea 2, 3, 8, 9, 21, 22, 24, 30, 50*, against *Fusarium nivale 1, 2, 8, 9, 13, 15, 18, 19, 21—24, 26, 36, 37, 47, 48, 51, 53—56*.

The results also document that the compound with the broadest inhibitory activity was *IV-9*, active against four organisms, compounds *2, 8, 21, 22* were active against three and compounds *15, 24, 36* against two species of microorganisms.

Quantitative assay of antifungal activity revealed that the most active against *Alternaria species* were compounds *2, 8, 21, 22*, against *Botrytis cinerea 2, 8, 21,*

22, *24*, and against *Fusarium nivale* compounds *13* and *26*. The last mentioned compounds were found to have the highest antifungal activity of all the compounds from the series *IV* Condensed isoxazolines were also tested for possible contact and systemic effect on *Erysiphe graminis* and *Phytophthora infestans*; compounds *3*, *6*, *10*, *12*, *36*, *37*, *39*, *41*—*45*, *50*, *52*, *55*, and *56* showed acaricidal, compounds *3*, *10*, *11*, and *41* retardant, and *38* auxinoid effect.

Experimental

Melting points were determined with a Kofler hot-stage. ^{1}H NMR spectra were taken with the spectrometer BS 487 C (Tesla) (80 MHz), ^{13}C NMR spectra with a spectrometer, model JX-100 (Jeol) of hexadeuteroacetone solutions with tetramethylsilane as internal standard, unless otherwise stated. Ultraviolet spectra of methanolic solutions were measured with the spectrophotometer M-40 (Zeiss, Jena) equipped with thermostated cells. Values of ε are given in $m^2 mol^{-1}$ and of λ in nm. The reactions were monitored by TLC chromatography on silufol (Lachema, Brno), containing fluorescence indicator (254 nm). Elemental analysis data never deviated more than by 0.4 % from the calculated values. The requisite phenylmaleinimides were obtained by the reaction of substituted anilines with malein anhydride [10].

The antimicrobial activity was tested on selected representatives of gram-positive (*Bacillus subtilis* CCM 1999, *Sarcina subflava* SMR 6/63, *Staphylococcus aureus* CCM 2022), gram-negative (*Proteus mirabilis* CCM 1944, *Escherichia coli* CCM 5172, *Salmonella typhimurium* TA 98) bacteria, on yeasts (*Candida albicans* CCY 29-3-112, *Saccharomyces cerevisiae* RIVE 10-25-8) and on phytopathogenic moulds (*Phytophthora infestans*, *Alternaria species* CCM 938, *Botrytis cinerea* CCM 16, and *Fusarium nivale* CCM 570). The bacteria were cultivated on the beef extract No. 2 (Imuna, Šarišské Michaľany), yeasts on the Sabourod soil (Imuna, Šarišské Michaľany) and synthetic VITA medium [11], the moulds on 2 % wort agar (Imuna, Šarišské Michaľany).

Cycloadducts IV-1—IV-56

Triethylamine (0.013 mol), dissolved in 25 cm^3 of dry ether, was during 1 h under stirring added to an ethereal solution (40 cm^3), containing dipolarophile *II* (0.01 mol) and *N*-hydroxybenzenecarboximidoyl chloride (0.01 mol), at −5 °C. The mixture was then stirred for 24 h at laboratory temperature, from the separated solid Et_3NHCl was thoroughly washed out with water. The crude product was purified either by crystallization or by column chromatography on alumina.

Antimicrobial screening

The antibacterial and anti-yeast activity was assessed by screening of a series of dimethyl sulfoxide solutions of the prepared compounds with concentrations in the range

Table 3

Antimicrobial effects of some studied isoxazolines and potassium sorbate (S) against *Saccharomyces cerevisiae* characterized with ED_{50} values estimated after 18 or 22 h cultivation

Compound *IV*	$ED_{50}/(\mu g\,cm^{-3})$	
	18 h	22 h
1	>65.6	>65.6
15	>65	>65
16	>72	>72
17	>72	>72
18	>74	>74
19	>74	>74
20	13.7	17.25
21	53.85	>67.8
22	>72	>72
23	>78.8	>78.8
24	16.1	22.75
29	>77.95	>77.95
S	>30	>30

Table 4

Minimal inhibitory concentrations of compounds (MIC)

Compound *IV*	A	B	C	D	E	F	G	H
21	1	0	1	1	1	1	4	4
35	0	0	1	0	0	0	1	1
39	0	0	1	1	1	1	2	2
46	0	0	1	0	1	1	0	2
47	0	0	1	0	1	0	2	3
54	0	0	1	0	1	1	2	3

A — *Bacillus subtilis*, B — *Staphylococcus aureus*, C — *Sarcina subflava*, D — *Proteus mirabilis*, E — *Escherichia coli*, F — *Salmonella typhimurium*, G — *Candida albicans*, H — *Saccharomyces cerevisiae*. The numbers represent concentrations/$(mol\,dm^{-3})$: 10^{-3}, 5×10^{-4}, 2.5×10^{-4}, 1.25×10^{-4}, 6.125×10^{-5}, respectively.

(10^{-3}—6.125×10^{-5}) $mol\,dm^{-3}$ (Tables 3 and 4). The compounds were first dissolved in DMSO and the solution diluted to the final concentration by the corresponding growth medium. The thus prepared solutions were sampled into the dents of a microtitration plate by automatic pipette (0.1 cm^3 per dent). The cultures grown on slant agar were later inoculated on a liquid medium and cultivated on a laboratory shaker at 37 °C for 5—6 h (bacteria and *C. albicans*), or at 24 °C for 18—24 h (*S. cerevisiae*). The inocula were prepared by diluting the medium with the requisite cultivating soil or physiological

solutions (*C. albicans*) so that their absorbance, measured with a Lange photocolorimeter, model VIII, was 0.05—0.07 in 1 cm cell. Under such conditions the cell count was $10^6/cm^3$ The filled microtitration plates were then kept at 37 °C resp. 24 °C in a thermostat. After 24 h (bacteria) or 48 h (yeasts) cultivation the turbidity was assessed by Microplate reader MR 700, as well as visually by magnifying mirror.

The inhibitory effect was quantified as MIC (minimal inhibitory concentration) values, which represent concentrations at which 100 % growth inhibition occurred. Such MIC values correspond to concentrations of tested compound in the nonturbid solution in the last dent. For *S. cerevisiae* also ED_{50} values were determined, that is concentration required for a 50 % decrease of growth intensity (relative to control samples). Absorbance of solutions placed in standard L-shaped test tubes was measured by the Lange colorimeter. The tubes contained the VITA medium, inoculated by *S. cerevisiae* cells so that there were eventually 1.5×10^6 cells per cm^3 Inoculation took place at 28 °C on a reciprocal shaker. Based on the measured values of absorbance growth curves were drawn, from which ED_{50} values were determined by graphical extrapolation after 10 and 22 h growth period, respectively.

Fungicidal activity against selected phytopathogenic fungi was determined by the diffusion method as follows: On a sterile paper (Whatman No. 3, diameter 10 mm) 10 mm^3 of solution of the tested compound was applied, *i. e.* 100 μg of active compound. After the solvent evaporated (acetone or other suitable solvent), the paper was placed on a sterile Petri dish ($d = 10$ cm), covered by the suspension of test organisms in optimal growth medium (2.5 cm^3 of aqueous suspension of spores and 10 cm^3 of agar medium kept at 45 °C). *Alternaria species* and *Botrytis cinerea* was cultivated on wort agar, *Fusarium* on glucose, and *Phytophthora* on rye agar. The spore inoculum was prepared from a two-week culture of fungus (*A. species*, *B. cinerea*, *Ph. infestans*), or a 21-day culture grown on slant agar (*Fusarium*), transferred by inoculation needle to 25 cm^3 of sterile distilled water. After homogenization and filtration through gauze the inoculum was diluted to contain 25×10^4 spores per cm^3

After the agar medium solidified, the dishes were thermostated for 72—96 h at a temperature optimal for the growth of the given pathogen (*A. species* and *B. cinerea* at 24 °C, *F. nivale* at 21 °C, *Ph. infestans* at 15 °C for the first 24 h, later all cultures at 18 °C) and the diameter of sterile zone formed around the centre of diffusion was measured.

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