Research on antibacterial and antifungal agents. XI. Synthesis and antimicrobial activity of N-heteroaryl benzylamines and their Schiff bases

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Summary — The synthesis and antimicrobial activity of new N-heteroaryl benzylamines and their Schiff bases are reported. Antifungal data were compared with those obtained with miconazole, ketoconazole, enilconazole and imazalil sulfate, which showed that some of the tested compounds possessed moderate activity against strains of *Candida albicans*, *Candida* sp and good activity against isolates of plant pathogenic fungi. In contrast the synthesized compounds showed poor antibacterial activity, except for **3j** which exhibited a better activity than nalidixic acid used as positive control. The results obtained are discussed on the basis of structure–activity relationships.

antifungal activity / N-heteroaryl benzylamine / agricultural fungicide / azole

Introduction

Agricultural fungicides include compounds that are characterized by widely diverse chemical structures and functional groups. They were often discovered and optimized without any knowledge of their biochemical targets. Various azoles (*eg*, imidazole, and 1,2,4-triazole) and heteroaryl derivatives (*eg*, pyridine, pyrimidine, piperazine, and quinoline) are active against plant pathogens [1]. The reasons for the efficacy of these diverse classes of active substances have not been completely explained. We have previously synthesized [2–6] many azole compounds that are active against *Candida albicans* and *Candida* sp, and have recently [2] tested many of our products against isolates of pathogenic plant fungi. We pointed out that 1-phenyl-2-(1H-triazol-1-yl) ethane derivatives were more active than the corresponding imidazole analogues. Compound **1** with R = F or CH₃, X = CHOH, Az = triazol-1-yl, showed a better activity *in vitro* at 6.25 μg/ml than imazalil sulfate or enilconazole used as positive controls. As a further extension of our previous research, we synthesized new com-

pounds **2** and **3** as a continuation of a structure–activity relationship (SAR) study on antifungal azole derivatives.
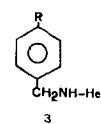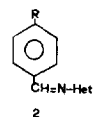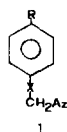
Compounds **1**. R = H; CH₃; Br; Cl; F. X = CHOH; –C=NOH. Az = imidazol-1-yl; triazol-1-yl.

Compounds **2** and **3**. R = H; CH₃; F; C₆H₅. Het = pyridin-2-yl; pyrimidin-2-yl; quinolin-3-yl; benzothiazol-2-yl; thiazol-2-yl; benzoimidazol-2-yl

The substituents in *para* position of the phenyl ring and the choice of the heterocyclic ring system were suggested by literature data [7–10] or by our previous research. In the present communication we report the
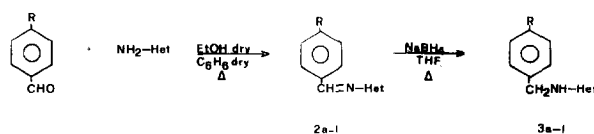
Scheme 1. R = H; CH₃; F; C₆H₅. Het = pyridin-2-yl, pyrim-idin-2-yl, quinolin-3-yl.

synthesis and *in vitro* antimicrobial activity against *C albicans, Candida* sp, Gram-positive and Gram-negative bacteria and isolates of some plant pathogenic fungi of the compounds **2** and **3** where Het = 2-pyridyl, 2-pyrimidyl and 3-quinolyl.

Chemistry

The synthetic pathways for the synthesis of the title compounds are illustrated in scheme 1. The Schiff bases **2b, 2d, 2i, 2j** and **2l** were prepared as previously de-

scribed *(Method A)* [11]. In contrast compounds **2a, 2c, 2f** and **2k** were obtained by melting the appropriate benzaldehyde and heteroarylamine *(Method B)*. Compounds **3a–e** were prepared from the corresponding Schiff bases by reduction with NaBH₄ in THF.

The structures of compounds **2a–l** and **3a–l** were confirmed by elemental analyses and spectral data. Electron impact mass spectra of some of the synthesized compounds have recently been reported [12].

Chemical and physical data of the compounds **2a–l** and **3a–l** are reported in tables I and II.

Results

The *in vitro* test results against *C albicans, Candida* sp, Gram-positive and Gram-negative bacteria and isolates of plant pathogenic fungi are given in tables III–XIII. Compounds **2b, 2c, 2f–g, 2j** and **3d–f** were found to be inactive against *C albicans* and *Candida* sp, while **3a–c, 3g** and **3l** were only inactive against *Candida* sp (tables III, IV). Derivatives **2a, 2i** and **2k** showed a poor activity against blastomycetes but only **3a** and **3l**

Table I. Chemical and physical data of the compounds 2a–l.

Compound	R	Het	mp (°C)	Yield (%)	Crystn solvent	Method	Formula (MW)
2a	H	2-Pyridyl	100–5	42	a	B	$C_{12}H_{10}N_2$ (182)
2b	CH₃	2-Pyridyl	80–3	55	a	A	$C_{13}H_{12}N_2$ (196)
2c	F	2-Pyridyl	88–90	45	a	B	$C_{12}H_9N_2F$ (200)
2d	Ph	2-Pyridyl	92–94	58	b	A	$C_{18}H_{14}N_2$ (258)
2e*	H	2-Pyrimidyl	220–3	46	a	B	$C_{11}H_9N_3$ (183)
2f	CH₃	2-Pyrimidyl	210–5	53	b	B	$C_{12}H_{11}N_3$ (197)
2g	F	2-Pyrimidyl	167–70	57	b	B	$C_{11}H_8N_3F$ (201)
2h	Ph	2-Pyrimidyl	199–202	75	c	B	$C_{17}H_{13}N_3$ (259)
2i*	H	3-Quinolyl	74–7	60	b	A	$C_{16}H_{12}N_2$ (232)
2j*	CH₃	3-Quinolyl	108–110	65	b	A	$C_{17}H_{14}N_2$ (246)
2k	F	3-Quinolyl	85–87	51	a	B	$C_{16}H_{11}N_2F$ (250)
2l*	Ph	3-Quinolyl	160–3	75	b	A	$C_{22}H_{16}N_2$ (308)

a = Cyclohexane; b = dry ethanol; c = benzene. *Literature [12].

Table II. Chemical and physical data of the compounds **3a–l.**

Compound	R	Het	mp ($^\circ$C)	Yield (%)	Crystn solvent	Formula (MW)
3a	H	2-Pyridyl	115–7	40	a	$C_{12}H_{12}N_2$ (184)
3b	CH_3	2-Pyridyl	79–81	50	a	$C_{13}H_{14}N_2$ (198)
3c	F	2-Pyridyl	94–8	40	a	$C_{12}H_{11}N_2F$ (202)
3d	Ph	2-Pyridyl	151–3	59	a	$C_{18}H_{16}N_2$ (260)
3e	H	2-Pyrimidyl	82–5	43	a	$C_{11}H_{11}N_3$ (185)
3f	CH_3	2-Pyrimidyl	118–20	49	a	$C_{12}H_{13}N_3$ (199)
3g	F	2-Pyrimidyl	90–4	60	a	$C_{11}H_{10}N_3F$ (203)
3h	Ph	2-Pyrimidyl	155–8	44	b	$C_{17}H_{15}N_3$ (261)
3i*	H	3-Quinolyl	96–8	60	b	$C_{16}H_{14}N_2$ (234)
3j*	CH_3	3-Quinolyl	131–3	48	a	$C_{17}H_{16}N_2$ (248)
3k	F	3-Quinolyl	93–5	57	a	$C_{16}H_{13}N_2F$ (252)
3l*	Ph	3-Quinolyl	193–4	49	a	$C_{22}H_{18}N_2$ (310)

a = Ethanol; b = cyclohexane. *Literature [12].

against *C albicans*. Compounds **2e**, **3g** and **3h** exhibited a weak activity against *C albicans*; derivatives **2h** and **3k** were moderately active against *C albicans* and *Candida* sp, while **3b–c** showed a weak or moderate activity only against strains of *C albicans*.

Data of tables III–IV clearly indicate that the most active compounds were **2d**, **2l**, **3i** and **3j**.

Regarding the antibacterial activity, all compounds were inactive except derivatives **2d**, **2h**, **2l**, **3i**, **3k** and **3l** as listed in table V. These compounds showed a moderate activity against Gram-positive bacteria. Among the synthesized compounds, the most active was **3j** against Gram-positive bacteria, which showed better activity than nalidixic acid used as a positive control.

The test results against isolates of some plant pathogenic fungi showed considerable *in vitro* activity for some of the tested compounds.

Drechslera graminea (Raben ex Schlecht) Shoem

The most active compounds were **2b** and **2d** which showed similar activity to the controls. Compounds **2f**, **3i** and **3k** exhibited good activity while **2h**, **2k** and

3f showed only a moderate inhibition. Compounds **2c**, **2g** and **3b** were not very active and all the other tested compounds showed a very poor inhibition (tables VI and VII).

Rhizoctonia solani Kühn

Compounds **2d**, **3b**, and **3i** were found to be the most potent compounds and their percentage of growth inhibition was comparable to the controls. Compounds **2b**, **3c** and **3f** showed a good activity while **2c**, **2g**, **3d** and **3g** were moderately active. All the other tested compounds showed a very poor inhibition (tables VIII and IX). Some compounds (*eg*, **3e** and **3h**) showed the same activity at 6.25 µg/ml as the controls but their activity was not enhanced by concentration.

Phomopsis sp

All tested compounds showed very poor activity with the exception of **2c**, **2d**, **2l** and **3c**, which exhibited moderate activity (tables X and XI).

Table III. Antimycotic activity of miconazole and ketoconazole compared with activity of the compounds **2b–d**, **2f**, **2g**, **3b–d** and **3f**, **3g** against 27 strains of *C albicans* and 6 strains of *Candida* sp* at pH 7.2.

Compound	Candida albicans			Candida sp.		
	R%	nX	Range (µg/ml)	R%	nX	Range (µg/ml)
2b	100		>256	100		>256
2c	100		>256	100		>256
2d	0	83	64-256	16	230	128->256
2f	100		>256			>256
2g	100		>256	100		>256
3b	18.5	256	256->256	100		>256
3c	11.1	14	8->256	100		>256
3d	100		>256	100		>256
3f	100		>256	100		>256
3g	48	256	256->256	100		>256
Miconazole	0	1.58	0.25-4	0	14.5	1-32
Ketoconazole	0	1.8	0.25-16	0	23	1-128

R%: percentage of resistant strains; nX: MIC mean values of sensitive strains; *1 *C parapsilosis*, 1 *C glabrata*, 1 *C tropicalis*, 1 *C krusei*, 1 *C lipolytica*, 1 *C stellatoidea*.

Table IV. Antimycotic activity of miconazole and ketoconazole compared with activity of the compounds **2a**, **2e**, **2h–l**, **3a**, **3e** and **3h–l** against 13 strains of *C albicans* and 19 strains of *Candida* sp* at pH 7.2.

Compound	Candida albicans			Candida sp.		
	R%	nX	Range (µg/ml)	R%	nX	Range (µg/ml)
2a	92.3	128	128->256	95	128	128->256
2e	89	256	256->256	35.3	234.2	16->256
2h	5.5	180.7	128->256	23.5	199.3	32->256
2i	92.3	256	256->256	95	16	6->256
2j	100		>256	95	8	8->256
2k	92.3	128	128->256	95	256	256->256
2l	0	140.3	32-256	25	221.8	128->256
3a	92.3	64	64->256	100		>256
3e	100		>256	100		>256
3h	66.6	160	64->256	94.1	256	256->256
3i	0	119.3	16-128	0	134.5	128-256
3j	0	32.6	8-64	20	85	16->256
3k	7.6	48.3	4-64	65	84.6	64->256
3l	84.6	128	128->256	100		>256
Miconazole	0	5.1	0.25-16	0	12.5	2-32
Ketoconazole	0	25.3	1-32	0	29.6	8-64

R%: percentage of resistant strains; nX: MIC mean values of sensitive strains; *4 *C tropicalis*, 4 *C torulopsis glabrata*, 1 *C guillermondii*, 3 *C paralopsis*, 5 *C krusei*, and 2 *C lipolytica*.

Botrytis cinerea Pers ex Fr

Compounds **2c**, **2d**, **3b**, **3c** and **3f** showed the same percentage of growth inhibition as imazalil sulfate at 6.25 µg/ml. Compounds **2b** and **2f** exhibited good activity, while **3d**, **3g** and **2l** showed only moderate inhibition (tables XII and XIII).

Discussion

From the microbiological data reported above, we can see that compounds **2b–d**, **2l**, **3b**, **3c**, **3i**, **3j** and **3l** are active against blastomycetes, plant pathogenic fungi and Gram-positive bacteria. Compounds **2b**, **2c**, **3b**, **3c** and **3l** show very good activity as inhibitors of plant

Table VII. Effects of compounds **2b–d**, **2f**, **2g**, **3b–d** and **3f**, **3g** on radial growth of *D graminea* isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2b	8	50	0	100	0	100	0	100	0	100
2c	8	50	8	50	8	50	8	50	0	100
2d	8	50	0	100	0	100	0	100	0	100
2f	8	50	8	50	0	100	0	100	0	100
2g	8	50	8	50	8	50	8	50	0	100
3b	11	31.2	11	31.2	8	50	8	50	0	100
3c	11	31.2	8	50	8	50	8	50	8	50
3d	15	6.25	12	25	11	31.2	8	50	8	50
3f	8	50	8	50	8	50	0	100	0	100
3g	11	31.2	11	31.2	10	37.5	8	50	8	50
Imazalil sulfate	8	50	0	100	0	100	0	100	0	100
Enilconazole	8	50	0	100	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 16 mm; the diameter of colonies in control treatments was 21 mm.

Table VIII. Effects of compounds **2a**, **2e**, **2h–l**, **3a**, **3e** and **3h–l** on radial growth of *R solani* isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2a	47	0	42	10.6	38	19.1	32	31.9	30	36.1
2e	46	2.1	38	19.1	35	25.5	24	48.9	18	61.7
2h	33	29.8	30	36.1	25	46.8	21	55.3	11	76.6
2i	35	25.5	29	38.3	26	47.7	18	61.7	15	68.1
2j	43	8.5	42	10.6	40	15.9	36	23.4	25	46.8
2k	31	34.0	28	40.4	18	61.7	17	63.8	16	65.9
2l	47	0	46	2.1	39	17.0	36	23.4	34	27.6
3a	30	36.1	28	40.4	24	48.9	22	53.2	21	55.3
3e	28	40.4	27	42.5	27	42.5	25	46.8	24	48.9
3h	28	40.4	27	42.5	25	46.8	21	55.3	16	65.9
3i	32	31.9	28	40.4	20	57.4	11	76.6	0	100
3j	44	6.4	42	10.6	36	23.4	35	25.5	33	29.8
3k	33	29.8	25	46.8	23	51.1	18	61.7	15	68.1
3l	30	36.1	27	42.5	23	51.1	22	56.8	20	57.4
Imazalil sulfate	37	21.3	23	51.1	16	65.9	11	76.6	8	83.0
Enilconazole	28	40.4	28	40.4	24	48.9	8	83.0	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 47 mm; the diameter of colonies in control treatments was 81 mm.

amine derivatives **3** are more active than the corresponding **2** against *R solani* with the sole exception of **2d**. Finally, the Schiff bases **2** are more active against *D graminea* than the corresponding amine **3**, with the sole exception of **3i**.

The heteroaryl ring system affects the antifungal activity against blastomycetes. We could state that the activity was decreasing in the order quinoline >> pyridine = pyrimidine. On the contrary, it is not possible to explain the influence of the heteroaryl ring system

Table IX. Effects of compounds **2b–d**, **2f**, **2g**, **3b–d** and **3f**, **3g** on radial growth of *R solani* isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2b	16	42.8	13	53.6	12	57.1	10	64.3	0	100
2c	27	3.6	18	35.7	11	60.7	8	71.4	8	71.4
2d	8	71.4	8	71.4	8	71.4	0	100	0	100
2f	19	32.1	12	57.1	11	60.7	11	60.7	11	60.7
2g	16	42.8	13	53.6	13	53.6	12	57.1	8	71.4
3b	8	71.4	8	71.4	0	100	0	100	0	100
3c	12	57.1	11	60.7	8	71.4	0	100	0	100
3d	18	35.7	11	60.7	11	60.7	8	71.4	8	71.4
3f	12	57.1	11	60.7	8	71.4	8	71.4	0	100
3g	24	14.3	17	39.3	14	50.0	8	71.4	0	100
Imazalil sulfate	8	71.4	0	100	0	100	0	100	0	100
Enilconazole	8	71.4	0	100	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 28 mm; the diameter of colonies in control treatments was 48 mm.

Table X. Effects of compounds **2a**, **2e**, **2h–l**, **3a**, **3e** and **3h–l** on radial growth of *Phomopsis* sp isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2a	37	2.6	36	5.3	35	7.9	34	10.5	32	15.8
2e	36	5.3	35	7.9	32	15.8	28	26.3	29	23.7
2h	28	26.3	25	34.2	22	42.1	20	47.4	19	50.0
2i	27	28.9	25	34.2	24	36.8	22	42.1	19	50.0
2j	32	15.8	30	21.0	29	23.7	27	28.9	25	34.2
2k	27	28.9	25	34.2	22	42.1	19	50.0	14	63.1
2l	36	5.3	27	28.9	14	63.1	10	73.7	0	100
3a	33	13.1	32	15.8	32	15.8	30	21.0	22	42.1
3e	37	2.6	36	5.3	34	10.5	28	26.3	23	39.5
3h	34	10.5	32	15.8	32	15.8	27	28.9	24	36.8
3i	35	7.9	34	10.5	32	15.8	25	34.2	22	42.1
3j	29	23.7	27	28.9	25	34.2	24	36.8	21	44.7
3k	32	15.8	30	21.0	22	42.1	14	63.1	11	71.0
3l	27	28.9	24	36.8	21	44.7	20	47.4	12	68.4
Imazalil sulfate	26	31.6	15	60.5	13	65.8	0	100	0	100
Enilconazole	14	63.1	0	100	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 38 mm; the diameter of colonies in control treatments was 45 mm.

on the growth inhibition of the isolates. The presence of the pyridine nucleus influences the inhibitor action more than the pyridine or quinoline nucleus.

We could state that the activity was decreasing in the following way: for *B cinerea* and *R solani*, pyridine > pyrimidine > quinoline with the exception of **2l** and **3i** respectively; and for *Phomopsis* sp and *D graminea*, pyridine > pyrimidine = quinoline, except for **2l** and **3i**.

The differences observed in the activities of various synthesized compounds towards each fungal species do not allow us to correlate chemical structure with

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Table XI. Effects of compounds 2b–d, 2f, 2g, 3b–d and 3f, 3g on radial growth of *Phomopsis* sp isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2b	16	15.8	15	21.0	15	21.0	13	31.6	11	42.1
2c	16	15.8	16	15.8	16	15.8	16	15.8	0	100
2d	17	10.5	16	15.8	13	31.6	10	47.4	0	100
2f	18	5.3	16	15.8	16	15.8	14	26.3	14	26.3
2g	19	0	19	0	17	10.5	17	10.5	15	21.0
3b	19	0	17	10.5	15	21.0	13	31.6	11	42.1
3c	15	21.0	14	26.3	13	31.6	12	36.8	0	100
3d	18	5.3	17	10.5	17	10.5	14	26.3	14	26.3
3f	19	0	16	15.8	16	15.8	13	31.6	11	42.1
3g	19	0	19	0	18	5.3	17	10.5	14	26.3
Imazalil sulfate	8	57.9	0	100	0	100	0	100	0	100
Enilconazole	8	57.9	0	100	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 19 mm; the diameter of colonies in control treatments was 32 mm.

Table XII. Effects of compounds 2a, 2e, 2h–l, 3a, 3e and 3h–l on radial growth of *B cinerea* isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2a	32	0	32	0	31	3.1	30	6.2	28	12.5
2e	32	0	32	0	30	6.2	27	15.6	22	31.2
2h	27	15.6	23	28.1	20	37.5	19	40.6	19	40.6
2i	32	0	27	15.6	25	21.9	24	25.0	21	39.4
2j	30	6.2	29	9.4	28	12.5	27	15.6	25	21.9
2k	27	15.6	25	21.9	24	25.0	20	37.5	13	59.4
2l	32	0	25	21.9	19	40.6	10	68.7	0	100
3a	32	0	31	3.1	30	6.2	29	9.4	27	15.6
3e	32	0	26	18.7	25	21.9	21	34.3	18	43.7
3h	29	9.4	26	18.7	25	21.9	23	28.1	22	31.2
3i	32	0	32	0	29	9.4	24	25.0	22	31.2
3j	29	9.4	27	15.6	25	21.9	24	25.0	22	31.2
3k	32	0	22	31.2	21	34.3	12	62.5	11	65.6
3l	26	18.7	21	34.3	20	37.5	15	53.1	13	59.4
Imazalil sulfate	19	40.6	8	75.0	0	100	0	100	0	100
Enilconazole	15	53.1	8	75.0	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 32 mm; the diameter of colonies in control treatments was 34 mm.

antifungal efficacy. In fact, the different phylogenetic position occupied by the species used in our assays can account for a large morphological and physiological diversity among the organisms. This diversity can affect the factors that are determinants of selectivity, such as uptake, detoxification or activation of, or differential sensitivity to, antifungal compounds, as reported by Kuhn [14].

The obtained results suggest further *in vivo* research on plants, which is in progress.

Table XIII. Effects of compounds **2b–d**, **2f**, **2g**, **3b–d** and **3f**, **3g** on radial growth of *B cinerea* isolates at pH 5.6.

Compound	Concentration									
	6.25µg/ml		12.5µg/ml		25µg/ml		50µg/ml		100µg/ml	
	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib	rg (mm)	% inhib
2b	8	33.3	8	33.3	0	100	0	100	0	100
2c	0	100	0	100	0	100	0	100	0	100
2d	0	100	0	100	0	100	0	100	0	100
2f	8	33.3	0	100	0	100	0	100	0	100
2g	8	33.3	0	100	0	100	0	100	0	100
3b	0	100	0	100	0	100	0	100	0	100
3c	0	100	0	100	0	100	0	100	0	100
3d	8	33.3	8	33.3	0	100	0	100	0	100
3f	0	100	0	100	0	100	0	100	0	100
3g	10	16.7	8	33.3	8	33.3	0	100	0	100
Imazalil sulfate	0	100	0	100	0	100	0	100	0	100
Enilconazole	8	33.3	0	100	0	100	0	100	0	100

rg: radial growth on potato dextrose (Oxoid); the diameter of colonies in control treatments with acetone was 12 mm; the diameter of colonies in control treatments was 38 mm.

Experimental protocols

Chemistry

Melting points were uncorrected and taken on a Fischer–Jones apparatus. Infrared spectra (nujol mulls) were run on a Perkin–Elmer spectrophotometer 297. NMR spectra were recorded on a Varian EM 390 (90 MHz) spectrometer using deutero-acetone, deutero-chloroform and deutero-DMSO as solvents and TMS as an internal standard. Mass spectra were recorded using a VG-TRIO 1 quadrupole mass spectrometer with a direct insertion probe (P = 10⁻⁴ torr, T = 170°C). Micro-analyses of the compounds **3** were performed by A Pietrogrande, Padova, Italy. Carlo Erba aluminium oxide (I-III according to Brockmann) was used for chromatographic purification. Fluka stratocrom aluminium oxide plates with fluorescent indicator were used for thin-layer chromatography (TLC) to check the purity of all compounds.

General procedure for the synthesis of Schiff bases **2**

Method A. The appropriate heteroarylamine (0.017 mol) was added to a solution of the appropriate benzaldehyde (0.017 mol) in dry ethanol (100 ml), dry benzene (50 ml) and 2 drops of glacial acetic acid. The mixture was refluxed for 24 h and the water formed during the reaction was eliminated using a Dean–Stark apparatus. After the reaction, the evaporation of the solvent gave compounds **2b**, **2d**, **2i**, **2j** or **2l**, which were crystallized from the appropriate solvent (table I).
Method B. The appropriate benzaldehyde (0.017 mol) and heteroarylamine (0.017 mol) were fused for 3 h. After the reaction the solid residue was crystallized from dry ethanol (table I).

Compound **2a**: ¹H-NMR (CDCl₃): δ (ppm) 7.10–8.33 (m, unresolved, 9H, C₆H₅ and pyridine); 9.33 (s, 1H, CH). Compound **2e**: ¹H-NMR (DMSO-d₆): δ (ppm) 7.13–7.73 (m, unresolved, 8H, C₆H₅ and pyrimidine); 8.53 (d, 1H, CH). Com-

pound **2i**: ¹H-NMR (CD₃COCD₃): δ (ppm) 7.33–8.23 (m, unresolved, 10H, C₆H₅ and quinoline); 8.73–9.10 (unresolved, 2H, CH and H₂ quinoline).

General procedure for the synthesis of amines **3**

To a solution of compound **2** (0.004 mol) in THF (100 ml) was added NaBH₄ (0.040 mol), and the reaction was kept at 25°C for 4 h. The suspension was then evaporated under reduced pressure. The residue was treated with water and chloroform. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in chloroform and passed through an aluminium oxide column. The eluates were collected after TLC control and the solvent removed to afford **3a–l**, which were recrystallized from ethanol (table II).

Compound **3a**: ¹H-NMR (CDCl₃): δ (ppm) 4.43 (d, 2H, CH₂, J = 6 Hz); 5.17 (br, 1H, NH); 6.16–7.96 (m, 9H, C₆H₅ and pyridine). Compound **3e**: ¹H-NMR (CDCl₃): δ (ppm) 4.60 (d, 2H, CH₂, J = 6 Hz); 6.33 (br, 1H, NH); 7.13–8.20 (m, 8H, C₆H₅ and pyrimidine). Compound **3i**: ¹H-NMR (CD₃COCD₃): δ (ppm) 4.40 (d, 2H, CH₂, J = 6 Hz); 5.90 (br, 1H, NH); 6.60–8.60 (m, 11H, C₆H₅ and quinoline).

Microbiology

Derivatives **2a–l** and **3a–l** were tested for *in vitro* antifungal activity against *C albicans* and various strains of *Candida* sp. Antibacterial activity against Gram-positive and Gram-negative bacteria was also investigated. Miconazole, ketoconazole and nalidixic acid were used as positive controls for antifungal and antibacterial activities. Minimum inhibitory concentration (MIC) was determined using the method of progressive double dilution in solid media [15]. Data were recorded after 36 h (fungi) or 24 h (bacteria); incubation was performed at 37°C. The substances were dissolved in DMSO (10 mg/ml); further dilution in the test medium furnished the required concentration, generally in the range of 0.2–200 µg/ml. The cultures were

obtained on Sabouraud (BBL) for fungi and BHI (BBL) for bacteria after 18 h incubation at 37°C. Tests were carried out using Sabouraud agar (BBL) with 70 µg/ml sodium dodecyl sulfate (SDS) and Muller-Hinton agar (Merck) with 70 µg/ml SDS; inocula were 10^7 for bacteria and 10^8 for *Candida*. Media MIC values (nX) and R% were calculated as previously reported [15]. Thirteen strains of *C albicans* and 19 strains of *Candida* sp (4 *C tropicalis*, 4 *C torulopsis glabrata*, 1 *C guillermondii*, 3 *C parapsilosis*, 5 *C krusei*, and 2 *C lipolytica*) were used to test compounds **2a**, **2e**, **2h–l**, **3a**, **3e**, and **3h–l**. Twenty-seven strains of *C albicans* and six strains of *Candida* sp (1 *C parapsilosis*, 1 *C glabrata*, 1 *C tropicalis*, 1 *C krusei*, 1 *C lipolytica* and 1 *C stellatoidea*) were used to test compounds **2b–d**, **2f**, **2g**, **3b–d** and **3f**, **3g**. Moreover, for all compounds the following species of bacteria isolated from various clinical specimens were tested: 15 Gram-positive (1 *Enterococcus cloacae*, 4 *E faecalis*, 5 *Streptococcus agalactiae*, 1 *Streptococcus* sp, 4 *Staphylococcus aureus*) and 16 Gram-negative (1 *Klebsiella oxytoca*, 1 *K pneumoniae*, 1 *Serratia odorifera*, 1 *Morganella morganii*, 4 *Pseudomonas aeruginosa*, 8 *Escherichia coli*).

The evaluation of the inhibitory activity on mycelial radial growth of plant pathogenic fungi isolates was carried out as previously reported [1]. *D graminea*, *Phomopsis* sp, *B cinerea* and *R solani* isolates were used for this assay. The isolates used were supplied by Istituto Sperimentale per la Patologia Vegetale, Roma. Imazalil sulfate (Janssen code N 009934), enilconazole (Janssen code N 024336), **2a–l** and **3a–l** were dissolved in acetone (5 mg/ml). Further dilution in the test medium furnished the required concentration in the range 6.25–100 µg/ml. The cultures were obtained on potato dextrose agar (Oxoid) at pH 5.6. Data were recorded after 72 h at 22°C. The activity of the compounds was estimated on the basis of percentage of growth inhibition by comparing the diameter of the zone of mycelial growth with that of the reference control with acetone.

Acknowledgments

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