Antifungal Agents, Part 11[1]

Biphenyl Analogues of Naftifine: Synthesis and Antifungal Activities

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Summary

A series of naftifine analogues having the biphenyl instead of the naphthyl moiety have been synthesized in a search devoted to study bioanalogues of clinically efficacious antifungal agents. The new derivatives were tested against *Candida albicans* by the direct contact method. They were also assayed against Gram-positive and Gram-negative bacteria and against some isolates of plant pathogenic fungi. Derivatives **8a**, **8c**, and **9a** were found to be active against *Candida albicans*, derivative **5a** was active against *E. coli*, a very resistant species to antimycotic agents, and derivatives **8a** and **8b** inhibited the plant pathogenic *Rhizoctonia solani*.

Naftifine (**1**)[2–4] and terbinafine (**2**)[5] are the most representative members of the allylamine class of antimycotics. Recently, new compounds related to above derivatives, *e.g.* butenafine (**3**)[6] and SDZ-87,469 (**4**)[7] have been prepared and tested against a wide range of pathogenic fungi.

Derivatives **1–4** lack the characteristic structural features of the azole antimycotics and act at a different stage of the biosynthetic pathway leading to steroids of antifungal membrane[2,8]. A naphthalene moiety and/or an allylamine chain characterize the chemical structure of this new type of antifungal agents.

Numerous studies[9–14] have shown that naftifine is a very efficacious topical antimycotic against various types of dermatomycoses. These results prompted study of the extent to which the antifungal activities of compounds **1-4** are specifically linked to their molecular structure and better definition of the structure-activity relationships of this new type of antifungal agents.

In previous work[15] on naphthyl analogues of bifonazole we observed that naphthyl and biphenyl moieties are bioisosteres and their mutual replacement could retain the initial biological activities. Furthermore, the biphenyl group is responsible for the potent antifungal activities of bifonazole (**6**), one of the most important antimycotic drugs used in clinical practice[16]. Bearing this in mind, we decided to prepare some biphenyl analogues of naftifine corresponding to the general formula **5**.

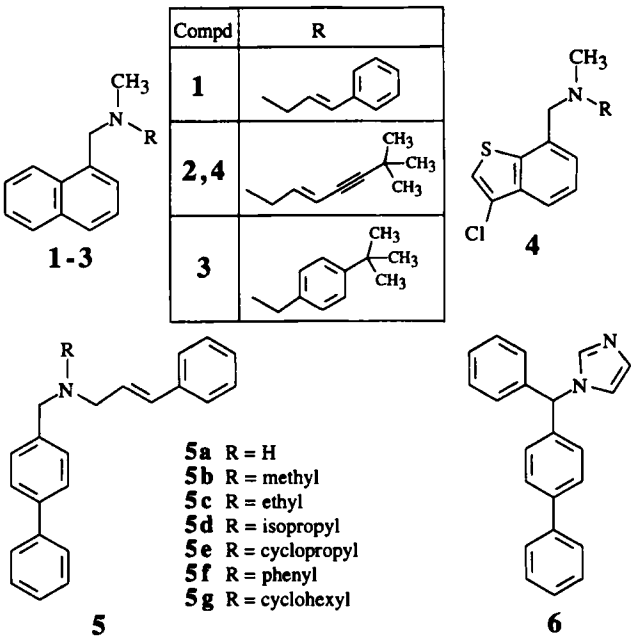

Figure 1. Naftifine (**1**), bifonazole (**6**), and related compounds.

Chemistry

The biphenyl analogue of naftifine and its *N*-ethyl homologue have been prepared by *N*-alkylation of *N*-(4-biphenylmethyl)-3-phenylallylamine, obtained by $NaBH_4$ reduction of the Schiff base **7** formed on reaction of 4-biphenylmethylamine with cinnamaldehyde. 4-Biphenylmethylamine has been prepared from 4-biphenylmethyl bromide *via* the Delepine procedure.

Derivatives **5d–g** were synthesized starting from 4-biphenylcarboxyaldehyde, which was allowed to react with appropriate amines to give the corresponding Schiff bases **8a–d**. Reduction of the azomethine linkage by $NaBH_4$ led to secondary amines **9a–d**, which were subjected to *N*-alkylation with phenylallyl bromide to afford the required tertiary allylamines **5d–g** (Scheme 1).

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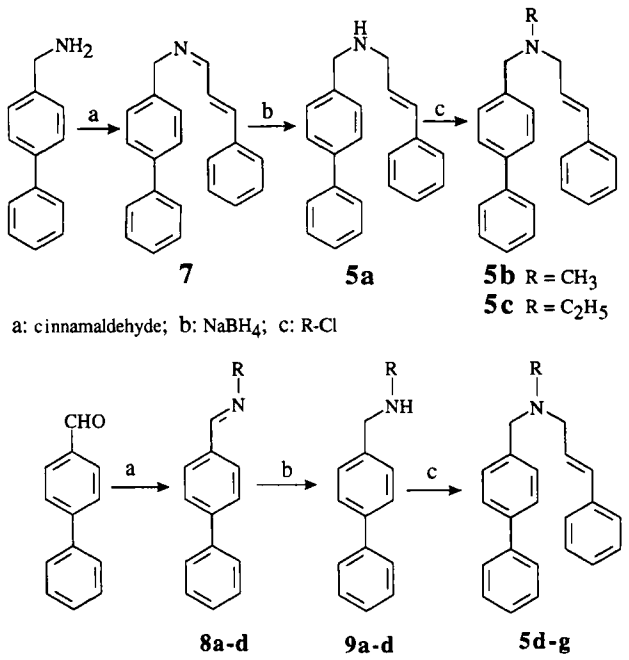

Scheme 1. Synthesis of **5a–g**.

Results and Discussion

The *in vitro* results of tests against *Candida albicans*, Gram-positive and Gram-negative bacteria as well as isolates of plant pathogenic fungi are given in Tables 1–4.

The antifungal activities of the new naftifine-like derivatives **5a-g**, and their intermediates **7**, **8a-d** and **9a-d** have been essayed by the contact test[19–21] in comparison with naftifine (**1**) and bifonazole (**6**). We choose the short term contact test for antifungal assays because it experimentally reproduces the real conditions of drug application in topical treatment of superficial mycoses better than the classical method of minimum inhibitory concentrations (MICs). The short term contact method analyzes the antifungal activity of a substance when it is put in contact with microorganisms for a short time and generally at high concentrations. It has been evidenced by previous studies[19–21] on the action of miconazole against *Candida albicans* that during the short term contact the active substance exerts its activity directly on the cellular membrane of microorganism thus determining alterations in its structure and permeability.

From the data of Table 1 it is evident that bifonazole and naftifine show a similar activity. Their mycocidal action was not very fast and a contact time of 30 min was needed for 50% killing (52.8% and 53.3% for naftifine and bifonazole, respectively) in the essay against *Candida albicans* 282. In the same test against *Candida albicans* 213 a 30% killing (29.5% and 32.1% for naftifine and bifonazole respectively) was observed after 30 min of contact.

Among the new test substances only **8a** behaves like naftifine and bifonazole with regard to the mycocidal action against both the strains used for the short time contact test.

Table 1: Activity of compounds **5a-g, 7, 8a-d, 9a-d,** naftifine and bifonazole against *Candida albicans* 282 and 213 at pH 7.2

Candida albicans 282

Tested [a] substance	Percentage of UFC survivors at 1 min	5 min	15 min
5a	94.2	87.8	84.5
5b	99.4	99.0	97.7
5c	97.0	89.3	88.3
5d	97.1	93.0	91.4
5e	99.0	97.2	96.2
8b	98.0	96.4	80.0
9a	98.8	77.1	70.4
9b	95.7	93.7	85.7
9c	94.8	92.1	90.0
9d	93.2	83.2	80.0
Naftifine	98.5	94.3	66.6
Bifonazole	99.0	98.5	64,3
5f	98.4	92.5	82.4
5g	97.3	86.2	72.5
7	94.5	92.3	91.8
8a	98.7	91.2	64.5
8c	89.8	87.5	78.4
8d	95.5	92.2	89.6
Naftifine	92.2	78.5	63.2
Bifonazole	91.4	84.2	62.8

Candida albicans 213

Tested [a] substance	Percentage of UFC survivors at 1 min	5 min	15 min
5a	98.2	97.2	90.5
5b	88.6	86.5	83.2
5c	91.5	88.9	83.0
5d	99.2	88.4	87.2
5e	99.6	91.4	90.0
8b	99.2	96.0	80.3
9a	93.9	83.6	79.4
9b	95.4	82.5	80.8
9c	88.8	86.4	82.6
9d	93.2	83.2	80.0
Naftifine	96.8	81.4	76.0
Bifonazole	99.2	96.2	74.4
5f	98.9	82.4	73.2
5g	99.7	85.2	76.5
7	95.5	83.3	81.8
8a	81.7	68.5	62.3
8c	97.5	85.3	69.2
8d	97.8	82.2	75.5
Naftifine	91.5	72.4	57.2
Bifonazole	83.9	62.4	49.2

[a] Conc. used for the essay: 1 mg/ml.

Table 2. MIC (μg/ml) media values of nalidixic acid and compounds **5a-g, 7, 8a-d,** and **9a-d** against 9 strains of Gram+ bacteria and 12 Gram- bacteria at pH 7.2

Tested substance	Gram-positive bacteria [a]			Gram-negative bacteria [b]		
	R% [c]	*nX* [d]	Range	*R*% [c]	*nX* [d]	Range
5a	22.2	32	32 – >256	41	210	64 – >256
8a	33.3	128	128 – >256	100	–	>256
8b	66.6	256	256 – >256	100	–	>256
8c	66.6	256	256 – >256	100	–	>256
9b	0	192	64 – 256	16	256	256 – >256
9d	0	128	64 – 256	16	179	128 – 256
NAX [e]	0	135	64 – 256	8	14	2 – 256
Naftifine	100	–	> 256	100	–	> 256

[a] *4 Streptococcus agalactiae, 2 Enterococcusfaecalis, 2 Staphylococcus cohnii, 1 Staphylococcus aureus.*
[b] *9 Escherichia coli, 1 Klebsiella pneumoniae, 2 Pseudomonas aeruginosa.*
[c] Percentage of resistant strains.
[d] MIC mean values of sensitive strains.
[e] Nalidixic acid.

Table 3: Activity of compounds **5a,** naftifine, and bifonazole against *E. coli* at pH 7.2

Tested [a] substance	Percentage of UFC survivors at 3 min	15 min
5a	100	53.4
Naftifine	100	100
Bifonazole	100	100

[a] Conc. used for the essay: 1 mg/ml.

Other compounds were inactive or showed moderate activity. In particular **9a**, **5g** and **8c** against *Candida albicans* 282 and, respectively, **8a**, **8c** and **5f** against *Candida albicans* 213 showed activities about 10% lower than those of controls.

It is noteworthy that the highest mycocidal activity was associated with azomethines **8a** and **8c** and at a less extent with their reduced counterpart **9a**, whereas all naftifine-like allylamines **5a-g** were inactive (**5a-e**) or scarcely active (**5f** and **5g**). Hence, contrary to expectation, the replacement of 1-naphthyl by 4-biphenyl moiety in the structure of naftifine did not furnished active bioisosteres. However, the good activity shown by biphenylmethyl azomethines **8a** and **8c** could offer new perspectives in a search directed to discover new antifungal agents.

It must be also pointed out that after a contact time of 5 min most of the new derivatives showed mycocidal action superior to those exerted by controls. In fact, either against *Candida albicans* 282, or against *Candida albicans* 213, derivatives **9a**, **9d**, **5g**, and, respectively, **8a** showed per cent of UFC (Units Forming Colonies) survivor values inferior to those of bifonazole and naftifine. However, unlike the controls, a longer time of contact (15 min) did not significantly improve their activity.

The antibacterial activities of the new derivatives against Gram-positive and Gram-negative are reported in Table 2 (data for derivatives with *R*% = 100 not shown).

In general, the new derivatives were found to be inactive or scarcely active. The most potent compounds against Gram-positive bacteria were **9b** (*R*% = 0; *nX* = 192) and **9d** (*R*% = 0; *nX* = 128). The last compound showed also some activity against Gram-negative bacteria, with a degree of potency about twelve times inferior to that of nalidixic acid and slightly superior to that of naftifine.

In contact experiments compound **5a** showed a significant activity (53.4% of UFC survivors) against *E.coli*, a species very resistant to antimycotic agents (Table 3), as proved by the fact that both naftifine and bifonazole showed 100% of UFC survivors after contact for 15 min.

The results of tests against isolates of some plant pathogenic fungi are reported in Table 4 (only derivatives which reached almost 100% inhibition at 100 μg/ml are reported) in comparison with imazalil sulfate and enilconazole. Against *Phomopsis* sp. (data not shown) all of test compounds showed very poor activity. Data from a test against *Drechslera graminea* indicate a poor activity of the new compounds, with the exception of derivatives **8a**, **8b**, and **8d**, which exhibited a moderate activity at 50 μg/ml (enilconazole and imazalil sulfate were active at 6.25 μg/ml). Better results were obtained against *Rhizoctonia solani*. Derivative **5a** was found to be more potent than controls also at 6.25 μg/ml, the minimum concentration used in the assay. Also **8d** showed antifungal activities comparable to those exhibited by enilconazole and imazalil sulfate. Other compounds (**5b**, **8a**, and **8b**) were moderately active. Against *Botrytis cinerea* all of test compounds showed very poor activity with the exception of **8a** and **8b**. Both derivatives were found as potent as enilconazole and slightly inferior to imazalil sulfate.

Although the replacement of 1-naphthyl by the 4-biphenyl moiety in the structure of naftifine did not provide satisfactory results against *Candida albicans*, we can emphasize that the new allylamines described here are strong inhibitors of the growth of some plant pathogenic fungi. Actually, this behaviour could be foreseen whether one observes that derivatives **5a**, **8a**, **8b**, **8d** and enilconazole incorporate the allyl chain in their structure, which might account for the potent antifungal activity exerted by these compounds against pathogenic fungi of plants.

Table 4. Effects of compounds **5, 7, 8,** and **9** on radial growth of *Botrytis cinerea, Drechslera graminea,* and *Rhizoctonia solani* isolates at pH 5.6

Pathogenic plant fungi	Tested substance	Concentration (μg/ml) 6.25 rg[a] (mm)	6.25 % inhib	12.5 rg (mm)	12.5 % inhib	25 rg (mm)	25 % inhib	50 rg (mm)	50 % inhib	100 rg (mm)	100 % inhib
	5a	12	7.9	10	13.2	8	18.4	8	18.4	0	100
	8a	8	18.4	8	18.4	0	100	0	100	0	100
Botrytis	**8b**	14	3.1	8	18.4	0	100	0	100	0	100
cinerea	**8 c**	10	13.2	8	18.4	8	18.4	8	18.4	0	100
	8d	15	0	15	0	8	18.4	0	100	0	100
	Imaz[b]	0	100	0	100	0	100	0	100	0	100
	Enilc[c]	8	18.4	0	100	0	100	0	100	0	100
	5b	10	27.6	8	34.5	8	34.5	8	34.5	0	100
	5d	12	20.7	11	24.3	10	27.6	8	34.5	0	100
Drechslera	**8a**	10	27.6	10	27.6	8	34.5	0	100	0	100
graminea	**8b**	15	10.3	8	34.5	8	34.5	0	100	0	100
	8c	13	17.2	10	27.6	8	34.5	8	34.5	0	100
	8d	8	34.5	8	34.5	8	34.5	0	100	0	100
	9a	18	0	14	13.8	10	27.6	8	34.5	0	100
	9b	8	34.5	8	34.5	8	34.5	8	34.5	0	100
	9d	8	34.5	8	34.5	8	34.5	8	34.5	0	100
	Imaz	0	100	0	100	0	100	0	100	0	100
	Enilc	0	100	0	100	0	100	0	100	0	100
	5a	15	6.3	11	11.3	0	100	0	100	0	100
	5b	10	12.5	8	34.5	8	34.5	8	34.5	0	100
Rhizoctonia	**8a**	20	0	19	1.3	17	3.8	8	15.0	0	100
solani	**8b**	20	0	15	6.3	12	10.0	8	15.0	0	100
	8d	17	3.8	17	3.8	12	10.0	0	100	0	100
	Imaz	17	3.8	13	8.8	10	12.5	8	15.0	0	100
	Enilc	13	8.8	10	12.5	8	15.0	0	100	0	100

[a] Radial growth on Potato dextrose (Oxoid): diameter of colonies in control treatments with ethanol was 15 mm, 18 mm, and 20 mm, for *B. cinerea, D. graminea and* R. *solani,* respectively; diameter of colonies in control treatments without ethanol was 38 mm, 29mm, and 80 mm, respectively, for the same species.

[b] Imazalil sulfate.

[c] Enilconazole.

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Experimental Part

M.p. Koefler (uncorr.).– IR spectra (nujol mulls): Perkin Elmer 1310. ^{1}H-NMR spectra: Varian EM 390 (90 MHz, TMS).– Column chromatography: alumina Carlo Erba (II-III according to Brockmann).– TLC: Cards Alumina Fluka (plates with fluorescent indicator).– Org. extracts were dried over Na_2SO_4.– Evaporation of solvents under reduced pressure.– Oily compounds were analyzed after chromatographic purification; solids were recrystallized from absolute ethanol. All derivatives were analyzed for C, H, and N; microanalyses data were within ± 0.4% of the theoretical values. Microanalyses: Laboratories of Prof. A. Pietrogrande, University of Padova (Italy).– Chemical and physical data of compounds **5a–g, 7, 8a–d** and **9a–d**: Table 5.

Preparation of azomethines **8a–d** *and* **7**

A solution of 4-biphenylcarboxaldehyde (2.0 g, 0.011 mol) and the appropriate aminoderivative (0.01 mol) in dry ethanol (50 ml) and benzene (20 ml) was treated with few drops of glacial acetic acid and heated under reflux for 24 h. During this period water was removed using a Dean-Stark apparatus. Removal of solvents furnished a residue, which was purified by recrystallization from suitable solvent. Compound **7** was prepared as reported for derivatives **8** starting from 4-biphenylmethylamine and cinnamaldehyde.

Table 5. Chemical and physical data of derivatives **5a-g, 7, 8a-d,** and **9a-d**

No.	Formula	M.p. (°C)	Yield (%)	Chromat. system [a] (crystall. solvent)
5a	$C_{22}H_{21}N$	oil [b]	76	A
5b	$C_{23}H_{23}N$	oil	30	A
5c	$C_{24}H_{25}N$	oil	50	A
5d	$C_{25}H_{27}N$	oil	56	B
5e	$C_{25}H_{25}N$	oil	30	C
5f	$C_{28}H_{25}N$	95–97	50	C
5g	$C_{28}H_{31}N$	58–60	58	B
7	$C_{22}H_{19}N$	100–103	74	(D)
8a	$C_{16}H_{17}N$	88–90	60	(D)
8b	$C_{16}H_{15}N$	72–75	71	(D)
8c	$C_{19}H_{15}N$	157–159	96	(D)
8d	$C_{19}H_{21}N$	107–110	95	A
9a	$C_{16}H_{19}N$	oil	74	A
9b	$C_{16}H_{17}N$	oil	80	A
9c	$C_{19}H_{17}N$	89–90	50	A
9d	$C_{19}H_{23}N$	64–65	90	A

[a] A: Al_2O_3 – chloroform; B: Al_2O_3 – cyclohexane; C: Al_2O_3 – cyclohexane:benzene (1: 1); D: ethanol.
[b] All oily compounds were purified by chromatography.

Preparation of amines **9a–d** *and* **5a**

A solution of **8a-d** (0.01 mol) in anhydrous ethanol (100 ml) was treated with $NaBH_4$ (1.9 g, 0.05 mol) and heated at reflux for 4 h. Evaporation of the solvent gave a residue, which was dissolved in chloroform (100 ml). The org. solution was washed with water, dried and evaporated. The residue was purified by passing through an alumina column to give pure **9a-d.** Compound **5a** was prepared starting from **7** by $NaBH_4$ reduction as described for amines **9a-d.**

5a: ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 1H, NH), 3.30 (d, 2H, -CH_2, J = 6 Hz), 3.80 (s, 2H, -CH_2), 6.00–6.70 (m, 2H, -CH=CH-), 7.00–7.80 (m, 14H, aromatic protons).

Preparation of allylamines **5d-g** *and* **5b,c**

A solution of **9a–d** (0.01 mol) in anhydrous dioxane (30 ml) was added slowly to a suspension of NaH (55–65% in white oil; 0.3 g, 0.011 mol) in the same solvent (20 ml). After addition the solution was heated at 100 °C for 15 min and then cooled. A solution of cinnamyl bromide (1.9 g., 0.011 mol) in anhydrous dioxane (20 ml) was added dropwise to the suspension and the mixture was heated at 100 °C for 18 h while stirring. The solution was cooled, treated with NH_4Cl (50 ml of saturated aqueous solution) and extracted with chloroform (3 × 50 ml). The extracts were collected, dried, and evaporated to give a residue which was purified by passing through an alumina column. Derivatives **5b–c** were prepared from **5a** by alkylation following the procedure used for compounds **5d–g.**

5b: ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 3.10 (d,2H,-CH_2, J = 3Hz), 3.50 (s,2H,-CH_2), 6.10–6.50 (m, 2H, -CH=CH-), 6.80–8.10 (m, 14H, aromatic protons); **5c**: 1.0 (t, 3H, CH_3, J = 3 Hz), 2.56, (q, 2H, -CH_2, J = 6 Hz), 3.20 (d,2H,-CH_2, J = 6 Hz), 3.60 (s,2H,-CH_2), 6.00–6.60 (m, 2H -CH=CH-), 7.00–7.70 (m, 14H, aromatic protons); **5d**: 1.00 (d, 6H, $2CH_3$, J = 6 Hz), 2.96 (m unr, 1H,-CH), 3.20 (s, 2H, -CH_2), 3.56 (s, 2H, -CH_2), 6.10–6.50 (m, 2H, -CH=CH-), 6.80–8.10 (m,14H,aromatic protons); **5e**: 0.40–0.60 (m, 4H, $2CH_2$), 3.30 (d, 2H, -CH2, J = 3 Hz), 3.80 (s, 2H, -CH_2), 4.10 (m unr, 1H, -CH), 6.10–6.60 (m, 2H, -CH=CH-), 7.10–7.60 (m, 14H, aromatic protons); **5f:** 4.20(d, 2H, -CH_2, J = 3 Hz), 4.60 (s, 2H, -CH_2), 6.30–6.45 (m, 2H, -CH=CH-), 6.50–7.80 (m,19H,aromatic protons); **5g**: 0.60–1.30 (m, 11H, cyclohexyl), 3.20 (d, 2H, -CH_2, J = 6 Hz), 3.60 (s, 2H, -CH_2), 6.00–6.50 (m, 2H, -CH=CH-), 7.10–7.70 (m,14H, aromatic protons).

Microbiological Part
Materials and Experimental Procedures

Antifungal activity: The "direct contact" test was used for evaluation of the cytocidal activity exerted by the new substances and controls when they were placed in contact with two *Candida albicans* strains, *C. albicans* 282 and *C.albicans* 213, respectively in the absence of a culture medium. All test substances were initially dissolved in DMSO at a concentration of 10 mg/ml with addition of 1% Tween 80 and then diluted in buffer phosphate until the concentration of 1 mg/ml was reached.

The time of direct contact between the new compounds and microorganisms ranged from 1 to 15 min. The antifungal activity was evaluated using microbial suspension (2×10^7 cells/ml) in 0.002 M buffer phosphate at pH 7.2. After contact, suspensions were diluted (1:1000) to abate the residual activity of test substance and the new solutions were then inseminated in *Sabouraud* agar (BBL) using the triple strata technique. The Units Forming Colonies (UFC) were calculated in percentage of inhibition related to microbial suspension developed after 48 h at 37 °C. Bifonazole and naftifine were used as positive controls.

Antibacterial activity: The antibacterial activity against Gram-positive and Gram-negative bacteria was investigated using the minimum inhibitory concentrations (MICs) test. The cultures of bacteria were obtained on BHI (BBL) for bacteria after 18 h incubation at 37 °C. Tests were carried out in *Muller-Hinton* agar (Merck) and *Muller-Hinton* broth with 70 μg/ml SDS; inocula were 10^7 cells/ml for bacteria. Nalidixic acid was used as positive control for antibacterial activity. Media MIC value (nX) and R% were calculated as reported[17]. Naftifine was used as reference compound. 9 Strains of Gram-positive (4 *Streptococcus agalactiae*, 2 *Enterococcus faecalis*, 2 *Staphylococcus cohnii*, 1 *Staphylococcus aureus*) and 12 strains of Gram-negative (9 *Escherichia coli*, 1 *Klebsiella pneumoniae*, 2 *Pseudomonas aeruginosa*) bacteria were used to test the activity of compounds **5a–g, 7, 8a–d,** and **9a–d.**

For compound **5a** the antibacterial activity against *E.coli* TS 260 strain was evaluated using the same technique employed to test the antifungal contact activity. Test substance was used at 1 mg/ml concentration. The time of contact ranged from 3 to 15 minute. The bacterial suspension cotained 3×10^7 cells ml^{-1} in 0.002 M buffer phosphate at pH 7.2. After contact suspensions were diluted 10^3 times and then cultivated in *Muller Hinton* agar (BBL) using the triple layer technique for 24 h at 37 °C.

Antifungal activity against plant pathogenic fungi: The evaluation of the inhibitory activity on mycelial radial growth of plant pathogenic fungi isolates was carried out as previously reported[18]. *Drechslera graminea* (Raben.ex Schlecht) Shoemaker, *Phomopsis* sp., *Botrytis cinerea* (Pers ex Fr.) and *Rhizoctonia solani* Kuhn 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), **5a-g, 7, 8a-d,** and **9a-d** were dissolved in ethanol (5 mg/ml); further dilution in the test medium produced the required concentration in the range of 6.25–100 μg/ml. The cultures were grown 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 on the reference control with ethanol.

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