FUNGICIDAL ACTIVITY OF SOME o-NITROPHENYL-HYDRAZONES

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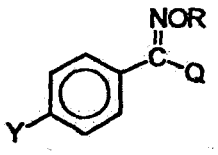
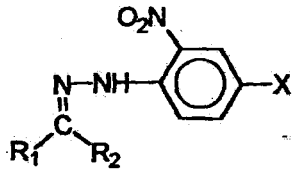
Summary — The antimycotic activity of 16 o-nitrophenylhydrazones against strains of *Hansenula anomala, Saccharomyces cerevisiae, Candida parapsylosis,* and *Cryptococcus albidus* was tested. All 16 compounds inhibited growth of the yeast strains. The inhibitory activity of the 4 methyl-derivatives substituted on the aromatic nucleus was particularly significant.

INTRODUCTION

The activity of nitrophenylhydrazones as inhibitors of the growth of some pathogenic microorganisms in plants has already been described[1,2]. More recently, other Authors[3] reported the effective fungicidal activity of o-derivatives of phenylpyridylamines with general formula (A), which are compounds similar to the o- nitrophenylhydrazones prepared by us (B).



A



B

Q = Pyridyl, Phenyl
X = H, CH_3, Cl, OCH_3
R_1 = H; R_2 = $(CH_2)_2$-CH_3, $(CH_2)_3$-CH_3
R_1 = CH_3; R_2 = CH_2-CH_3, $(CH_2)_2$-CH_3

These findings encouraged us to prepare and screen a series of 16 new o-nitrophenylhydrazones of aldehydes and aliphatic ketones for antimycotic activity.

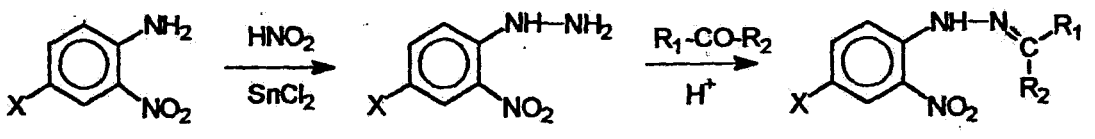
These assays (worked out at the Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, sez. Microbiologia, University of Sassari) provided some interesting results. All the prepared o-nitrophenylhydrazones assayed at concentrations ranging between 50 and 400 μg/ml showed inhibitory activity. Compounds **2, 6, 10** and **12** (Table I) were particularly active, being able to inhibit growing colonies at concentrations lower than 50 μg/ml.

A correlation between structure and inhibitory activity can be hypothesized. The linear chains R_2 (see Table I) were more active than the corresponding branched ones. Concerning the substituent X of the aromatic nucleus, the best pharmacological effects were obtained when CH_3 was present, whereas when Cl was present compounds were inactive.

CHEMISTRY

All products used in the tests were synthesized as shown in Scheme 1, starting from 4-substituted o-nitroanilines (C) which were transformed into hydrazines (D) and condensed with aldehydes and ketones to obtain the corresponding derivatives.

Yields were generally good. The prepared compounds are listed in Table 1. For all the unknown compounds the spectroscopic and mass data useful for their characterization are reported in Table II.



C D B

X = H, CH_3, Cl, OCH_3

R_1 = H; R_2 = $(CH_2)_2$-CH_3, $(CH_2)_3$-CH_3
R_1 = CH_3; R_2 = CH_2-CH_3, $(CH_2)_2$-CH_3

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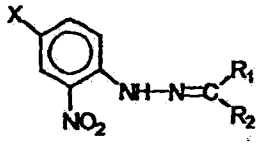
TABLE I - List of the prepared compounds



N°	X	R_1	R_2	yeld %	p.f. °C
1	H	H	$-(CH_2)_2-CH_3$	81	59-60
2	CH_3	H	$-(CH_2)_2-CH_3$	81	68-69
3	Cl	H	$-(CH_2)_2-CH_3$	74	63-64
4	OCH_3	H	$-(CH_2)_2-CH_3$	78	102-103
5[4]	H	H	$-(CH_2)_3-CH_3$	74	39-40
6	CH_3	H	$-(CH_2)_3-CH_3$	79	57-58
7	Cl	H	$-(CH_2)_3-CH_3$	82	71-72
8	OCH_3	H	$-(CH_2)_3-CH_3$	85	70-71
9[5]	H	CH_3	$-CH_2-CH_3$	78	73
10[1]	CH_3	CH_3	$-CH_2-CH_3$	93	81-82
11[1]	Cl	CH_3	$-CH_2-CH_3$	74	70-71
12	OCH_3	CH_3	$-CH_2-CH_3$	72	75-76
13[6]	H	CH_3	$-(CH_2)_2-CH_3$	83	51-52
14	CH_3	CH_3	$-(CH_2)_2-CH_3$	64	33
15[7]	Cl	CH_3	$-(CH_2)_2-CH_3$	76	82-83
16	OCH_3	CH_3	$-(CH_2)_2-CH_3$	68	54-55

Reference numbers are given for compounds already reported in the literature.

FUNGICIDAL ACTIVITY

The compounds were tested for inhibition of the growth of the pathogenic strains: 1) 201 *Hansenula anomala* 2) 1090 *Saccharomyces cerevisiae* 3) 2813 *Candida parapsylosis* 4) 3053 *Cryptococcus albidus.*

All strains were obtained from the Dipartimento di Scienze Agrarie e Biotecnologie Agro-Alimentari, sez. Microbiologia, University of Sassari.

Solutions of the products to be assayed were prepared in eight different concentrations (50, 100, 150, 200, 250, 300, 350 and 400 μg/ml). GYEP culture medium was used for fungal growth.

Blank and inhibition tests with a 50 μg/ml concentration of miconazole were also carried out, counting the Colony Forming Units (CFU) 48 h after having inoculated the culture medium. The results are shown in Tables III, IV, V and VI. They give the CFU expressed as exponential notations for each compound at each concentration (μg/ml). The data are summarized in Table VII.

At the maximum dose (400 μg/ml), the formation of yeast colonies was strongly inhibited. The growth reduction factor was calculated as follows:

$$100 \bullet \frac{(\text{CFU-comp.})-(\text{CFU}+\text{comp.})}{(\text{CFU-comp.})}$$

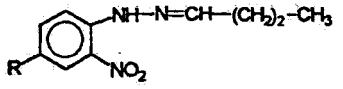
It was over 99.9% in almost all cases.

Table VII shows the concentrations in μg/ml which caused growth inhibition in the strains tested, giving a result between $0 \div 10^2$ CFU, compared with 10^9 in the blank test. Two (6 and 10) of the most active compounds were tested at concentrations between 5 and 25 μg/ml. The results are reported in Table VIII. Both

TABLE II - Physical and spectrometric properties of the new compounds

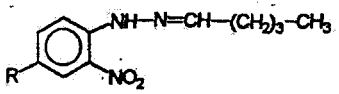
Comp.	Formula	M.W.	GC-MS M^+/z	IR (cm^{-1}) -NH C=N $-NO_2$	^{1}HNMR (δ) $CDCl_3$
1	$C_{10}H_{13}N_3O_2$	207.2	Compatible	3300–1630–1500 1340	1.00 (t, H, $-CH_3$); 1.60 (m, 2H $-CH_2-\underline{CH_2}-CH_3$); 2.37 (q, 2H $-\underline{CH_2}-CH_2-CH_3$); 7.33 (m, 5H, 4H arom. +1H methylenic); 10.70 (s, 1H, NH)
2	$C_{11}H_{15}N_3O_2$	221.2	Compatible	3300–1630–1500 1330	1.00 (t, H, $-CH_3$); 1.62 (m, 2H $-CH_2-\underline{CH_2}-CH_3$); 2.28 (s, 3H Ar-$CH_3$); 2.33 (q, 2H, $-CH_2-\underline{CH_2}-CH_3$); 7.36 (m, 4H, 3H arom. +1H methylenic); 10.64 (s, 1H NH)
3	$C_{10}H_{12}N_3O_2Cl$	241.7	Compatible	3300–1630–1500 1330	1.00 (t, 3H, $-CH_3$); 1.61 (m, 2H, $-CH_2-\underline{CH_2}-CH_3$); 2.34 (q, 2H, $-\underline{CH_2}-CH_2-CH_3$); 7.37-8.13 (m, 4H, 3H arom. +1H methylenic); 10.66 (s, 1H, NH)
4	$C_{11}H_{15}N_3O_3$	237.2	Compatible	3285–1630–1500 1320	0.99 (t, H, $-CH_3$); 1.59 (m, 2H $-CH_2-\underline{CH_2}-CH_3$); 2.32 (q, 2H, $-\underline{CH_2}-CH_2-CH_3$); 3.79 (s, 3H, Ar-$OCH_3$); 7.15-7.79 (m, 4H, 3H arom. +1H methylenic); 10.66 (s, 1H, NH)
6	$C_{12}H_{17}N_3O_2$	235.3	Compatible	3300–1630–1510 1330	0.94 (t, 3H, $-CH_3$); 1.41 (m, 2H, $-CH_2-$); 1.55 (m, 2H, $-CH_2-$); 2.28 (s, 3H Ar-CH_3);2.35 (q, 2H $-CH_2$); 7.34 (m, 4H, 3H arom. +1H methylenic); 10.64 (s, 1H,NH)
7	$C_{11}H_{14}N_3O_2Cl$	255.7	Compatible	3300–1620–1500 1330	0.94 (t, 3H, $-CH_3$); 1.41 (m, 2H, $-CH_2-$); 1.56 (m, 2H, $-CH_2-$); 2.37 (q, 2H, $-CH_2$); 7.37-8.13 (m, 4H, 3H arom. +1H methylenic); 10.66 (s, 1H, NH)
8	$C_{12}H_{17}N_3O_3$	251.3	Compatible	3290–1625–1495 1325	0.93 (t, H, $-CH_3$); 1.40 (m, 2H, $-CH_2-$); 1.55 (m, 2H, $-CH_2-$); 2.33 (q, 2H, $-CH_2-$) 3.79 (s, 3H, Ar-OCH_3); 7.16-7.78 (m, 4H, 3H arom. +1H methylenic); 10.66 (s, 1H, NH)
12	$C_{11}H_{15}N_3O_3$	237.2	Compatible	3315–1620–1510 1335	1.16 (t, H, $-CH_3$);1.98 (s, 3H, CH_3); 2.39 (q, 2H, $-\underline{CH_2}-CH_2-CH_3$); 3.81 (s, 3H, Ar-$OCH_3$);7.17-7.88 (m, 3H, arom.); 10.61 (s, 1H, NH)
14	$C_{12}H_{17}N_3O_2$	235.3	Compatible	3300–1630–1500 1330	0.99 (t, H, $-CH_3$); 1.64 (m, 2H $-CH_2-\underline{CH_2}-CH_2$); 1.98 (s, 3H, CH_3); 2.29 (s, 3H Ar-CH_3); 2.35 (t, 2H $-\underline{CH_2}-CH_2-CH_3$); 7.30-7.92 (m, 3H arom.); 10.59 (s, 1H, NH)
16	$C_{12}H_{17}N_3O_3$	251.3	Compatible	3310–1630–1500 1335	0.96 (t, 3H, $-CH_3$); 1.63 (m, 2H, $-CH_2-\underline{CH_2}-CH_3$); 1.97 (s, 3H CH_3);2.34 (t, 2H $-\underline{CH_2}-CH_2-CH_3$); 3.80 (s, 3H, Ar-$OCH_3$); 7.17-7.84 (m, 3H, arom.); 10.60 (s, 1H, NH)

TABLE III - Growth inhibitory activity of compounds **1**, **2**, **3** and **4**



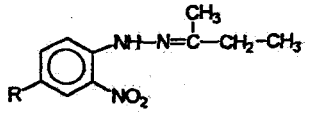
COMPOUND 1 (R = H)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	4.10^9	2.10^9	2.10^9	2.10	0	0	0	0	0
1090	6.10^9	4.10^9	8.10^8	4.10^4	6.10^3	0	0	0	0
2813	6.10^9	2.10^8	810^7	4.10	0	0	0	0	0
3053	2.10^9	4.10^3	0	0	0	0	0	0	0
COMPOUND 2 (R = CH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	4.10^9	4.10^4	6.10	0	0	0	0	0	0
1090	6.10^9	2.10^8	6.10^5	4.10^2	2.10	0	0	0	0
2813	6.10^9	6.10^3	4.10^2	6.10^2	0	0	0	0	0
3053	2.10^9	2.10^7	6.10^3	2.10^2	0	0	0	0	0
COMPOUND 3 (R = Cl)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	4.10^9	6.10^6	4.10^6	8.10^2	6.10^2	0	0	0	0
1090	6.10^9	4.10^9	4.10^9	6.10^5	6.10^5	4.10^5	6.10^4	2.10^4	8.10^3
2813	6.10^9	6.10^7	4.10^7	6.10^2	2.10^2	6.10^2	8.10	6.10	4.10
3053	2.10^9	6.10^3	4.10	2.10	0	0	0	0	0
COMPOUND 4 (R = OCH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	4.10^9	4.10^7	4.10	0	0	0	0	0	0
1090	6.10^9	4.10^9	4.10^7	6.10^4	2.10^3	8.10^2	6.10	0	0
2813	6.10^9	4.10^9	2.10^9	4.10^3	4.10^2	4.10	0	0	0
3053	2.10^9	6.10^4	2.10^2	0	0	0	0	0	0

TABLE IV - Growth inhibitory activity of compounds **5**, **6**, **7** and **8**



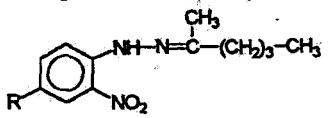
COMPOUND 5 (R = H)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^4	10^2	10^2	10^2	10^2	10^2	10
1090	10^9	10^8	10^5	10^3	10^3	10^3	10^3	10^3	10^3
2813	10^9	10^9	10^7	10^4	10^4	10^4	10^4	10^2	10^2
3053	10^9	10^9	10^5	10^8	10^7	10^6	10^6	10^5	10^3
COMPOUND 6 (R = CH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	0	0	0	0	0	0	0	0
1090	10^9	10^3	0	0	0	0	0	0	0
2813	10^9	10^4	10^2	0	0	0	0	0	0
3053	10^9	10^3	0	0	0	0	0	0	0
COMPOUND 7 (R = Cl)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^8	10^8	10^7	10^6	10^5	10^5	10^4
1090	10^9	10^9	10^9	10^6	10^6	10^6	10^5	10^4	10^3
2813	10^9	10^9	10^9	10^7	10^6	10^5	10^5	10^4	10^2
3053	10^9	10^9	10^9	10^8	10^8	10^6	10^5	10^5	10^5
COMPOUND 8 (R = OCH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^7	10^3	10^3	10^3	10^2	10^2	10^2	0
1090	10^9	10^9	10^6	10^4	10^4	10^4	10^2	10^2	0
2813	10^9	10^5	10^2	10^2	10^2	0	0	0	0
3053	10^9	10^5	10^4	10^4	10^3	10^2	10^2	10^2	0

TABLE V - Growth inhibitory activity of compounds **9**, **10**, **11** and **12**



COMPOUND 9 (R = H)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^5	10^3	10^3	10^2	10	10	10	10
1090	10^9	10^5	10^3	10^3	10^3	10^2	10^2	10^2	10^2
2813	10^9	10^5	10^5	10^2	10^2	0	0	0	0
3053	10^9	10^9	10^9	10^9	10^7	10^5	10^3	10^2	0
COMPOUND 10 (R = CH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	0	0	0	0	0	0	0	0
1090	10^9	10^3	0	0	0	0	0	0	0
2813	10^9	10^4	10^2	0	0	0	0	0	0
3053	10^9	10^3	0	0	0	0	0	0	0
COMPOUND 11 (R = Cl)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^6	10^5	10^5	10^5	10^4	10^3	10^3
1090	10^9	10^9	10^5	10^4	10^4	10^3	10^3	10^3	10^3
2813	10^9	10^6	10^4	10^3	10^3	10^2	10^2	10^2	10^2
3053	10^9	10^9	10^9	10^7	10^7	10^6	10^6	10^5	10^5
COMPOUND 12 (R = OCH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^4	10^2	10^2	10^2	10^2	10^2	10^2	10^2
1090	10^9	10^9	10^4	10^2	10^2	10^2	10^2	10^2	10^2
2813	10^9	10^6	10^2	10^2	10^2	0	0	0	0
3053	10^9	10^4	10^3	10^2	10^2	10^2	10^2	10	10

TABLE VI - Growth inhibitory activity of compounds **13**, **14**, **15** and **16**



COMPOUND 13 (R = H)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^8	10^5	10^4	10^3	10^2	10^2	10^2
1090	10^9	10^9	10^7	10^7	10^4	10^4	10^3	10^3	10^3
2813	10^9	10^5	10^4	10^4	10^4	10^4	10^4	10^2	10
3053	10^9	10^9	10^9	10^8	10^7	10^7	10^6	10^5	10^4
COMPOUND 14(R = CH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^4	10^4	10^3	10^2	10^2	10^2	10^2	10^2
1090	10^9	10^6	10^6	10^5	10^3	10^3	10^2	10^2	10^2
2813	10^9	10^5	10^3	10^3	10^2	10^2	10^2	10^2	10^2
3053	10^9	10^7	10^4	10^2	10^2	10^2	10^2	10^2	10
COMPOUND 15 (R = Cl)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^8	10^8	10^6	10^6	10^4	10^4	10^4
1090	10^9	10^9	10^9	10^8	10^7	10^6	10^5	10^5	10^4
2813	10^9	10^9	10^8	10^8	10^7	10^4	10^4	0	0
3053	10^9	10^9	10^5	10^5	10^4	10^3	10^3	10^2	10^2
COMPOUND 16 (R = OCH_3)									
YEASTS	CONCENTRATIONS (μg/ml)								
	0	50	100	150	200	250	300	350	400
201	10^9	10^9	10^9	10^8	10^7	10^6	10^5	10^5	10^5
1090	10^9	10^9	10^9	10^8	10^7	10^6	10^6	10^3	10^2
2813	10^9	10^9	10^8	10^8	10^5	10^5	10^3	10^2	10^2
3053	10^9	10^8	10^6	10^6	10^5	10^3	10^3	10^3	10^3

TABLE VII - Concentrations of compounds tested (μg/ml) producing less than 10^2 CFU after 48 h

Compounds	Yeasts tested			
	201 Hans. an.	3053 Sacc. cer.	2813 Cand. par.	1090 Crypt. alb
1	100	100	150	250
2	100	150	100	150
3	200	100	150	>400
4	100	100	200	250
5	150	>400	350	>400
6	<50	100	100	100
7	>400	>400	400	>400
8	250	300	100	250
9	200	250	150	350
10	<50	100	100	100
11	>400	>400	250	>400
12	100	150	100	150
13	300	>400	350	>400
14	200	300	200	150
15	>400	>400	350	350
16	>400	400	350	>400
Miconazole	<50	<50	<50	<50

TABLE VIII - Growth inhibitory activity of compounds **6** and **10** at concentrations of 5-25 μg/ml

COMPOUND 6				
YEASTS	CONCENTRATIONS (μg/ml)			
	0	5	15	25
201	3.10^9	2.10^9	8.10^7	3.10^4
1090	5.10^9	6.10^9	3.10^7	2.10^3
2813	2.10^9	5.10^6	3.10^6	6.10^3
3053	2.10^9	3.10^9	6.10^8	7.10^6
COMPOUND 10				
YEASTS	CONCENTRATIONS (μg/ml)			
	0	5	15	25
201	3.10^9	2.10^6	4.10^4	0
1090	5.10^9	5.10^6	6.10^5	2.10^5
2813	2.10^9	3.10^8	2.10^7	3.10^6
3053	2.10^9	6.10^6	3.10^5	7.10^4
MICONAZOLE				
YEASTS	CONCENTRATIONS (μg/ml)			
	0	5	15	25
201	0	0	0	0
1090	0	0	0	0
2813	0	0	0	0
3053	0	0	0	0

they have a CH_3 in 4 on the aromatic nucleus and a linear alkyl chain of 4 or 5 C atoms on hydrazone N; the best results were shown by compound 6 (CH_3 in 4 and linear chains of 5C).

It is probable that these substituents play an essential role in crossing the cell membranes of the yeasts, as confirmed by the relatively higher resistance to inhibition of *Saccharomyces cerevisiae* strain) 1090 which has thicker membranes than the other strains tested.

Future research will assess any other effects produced by longer (and therefore more lipophilic) alkyl chains inserted in the position 4 of the aromatic system. Our research will be extended to include inhibitory activity against bacterial strains pathogenic for human beings.

CHEMICAL EXPERIMENTAL SECTION

PREPARATION OF O-NITROPHENYLHYDRAZINES

The reaction was performed in alcohol bath at a temperature between -5 °C and 0 °C using 0.3 moles of the appropriate o-nitroaniline suspended in concentrated HCl (Scheme 1). The cooled suspension was slowly added with $NaNO_2$ solution (20 g in 60 ml of H_2O). Following, a $SnCl_2.2H_2O$ in HCl solution (125 g in 115 ml of conc. HCl) was dropped.

The resulting crude product was filtered under vacuum, washed with cold H_2O, and dissolved in boiling H_2O. To the filtered warm solution CH_3COONa was added. The precipitated o-nitrophenylhydrazine was filtered and dried. For each compound yield was about 60-65%.

PREPARATION OF O-NITROPHENYLHYDRAZONES

A solution of the appropriate o-nitrophenylhydrazine in ethanol (w/v 1:5-1:10) was added with 1-2 drops of conc. HCl and a stoichiometric quantity, plus a 10% excess, of the appropriate ketone or purified aldehyde. The hydrazone crystallized directly from the cooled alcohol solution and was recovered by filtration.

The products were analyzed by TLC and melting point determined using a Kofler apparatus. Molecular structures were assigned by IR spectroscopy ^{1}H-NMR and confirmed by GS-MS analysis (see Table 2).

MICROBIOLOGICAL EXPERIMENTAL SECTION

The following yeasts were used: strain 1090 of *Saccharomyces cerevisiae*, strain 201 of *Hansenula anomala*, strain 2813 of *Candida parapsylosis*, strain 3053 of *Cryptococcus albidus*. All

strains were obtained from the Dipartimento di Scienze Ambientali-Agrarie e Biotecnologie Agro.-Alimentari, sez. Microbiologia, University of Sassari.

GYEP culture medium (2% glucose, 0.5% yeast extract, 1% bacteriological peptone, 2% agar, in aqueous solution) was sterilized at 115°C for 30 min. Mother solutions of o-nitrophenylhydrazones in ethanol at a concentration of 10 mg/ml were prepared and added to the GYEP in order to obtain concentrations of 50, 100, 150, 200, 250, 300, 350, 400 μg/ml of o-phenylhydrazones. The solutions were then placed in Petri dishes and inoculated with eight different concentrations ($10-10^{-8}$) of appropriate precultures of yeast strain. Precultures of the yeast strains were grown in liquid GYEP (as above but without the agar). The same procedure was used for comparison with miconazole at a single concentration of 50 μg/ml. A blank test was also carried out, using the same culture medium without the addition of the tested compounds. Colony Forming Unit (CFU) counts were made after 48 h. The results are shown in Tables III, IV, V, and VI and summarized in Table VII.

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REFERENCES

(1) Kururay Co. Ltd., *Jpn. Kokai Tokio, 80,* 118, 434 (1980) *C.A. 94* 191702d (1981).
(2) M. Podova, J. Zemek, M. Valent, K. Linek, P. Demes, *Acta Univ. Carolinae, 30,* 547 (1986).
(3) G. Massolini, M.L. Carmellino, M. Kitsos, A. Baruffini, *Il Farmaco, 48,* 503 (1993).
(4) R. Cerri, G. Boatto, A. Pau, F. Sparatore, P. Manca, *Il Farmaco Ed. Sci., 43,* 113 (1988).
(5) H. Bauer, E. Strauss, *Ber., 65,* 308 (1932).
(6) Z. J. Vejedelek, *Chem. Listy, 51,* 1338 (1957) *C.A. 51,* 17874i (1957).
(7) R. Cerri, G. Boatto, A. Pau, F. Sparatore, L. Cima, M. Carrara, M. Satta, *Il Farmaco, 46,* 369 (1991).
(8) L. Sansebastiano, L. Mosti, G. Menozzi, P. Schenone, O. Muratore, A. Petta, E. Debbia, A. Pesce Schito, G. C. Schito, *Il Farmaco, 48,* 335 (1993).

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