Eur J Med Chem (1994) 29, 701–706

Short communication

Aliphatic ethers and esters of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl) ethanol: study of antifungal activity against yeasts and hydrophobic character

Y Wahbi[1], C Tournaire[1], R Caujolle[1]*, M Payard[1], MD Linas[2], JP Seguela[2]

[1]*Département de Chimie, Faculté de Pharmacie, 35, chemin des Maraîchers, 31400 Toulouse;*
[2]*Laboratoire de Parasitologie et Mycologie, CHU de Rangueil, 31054 Toulouse Cedex, France*

(Received 7 March 1994; accepted 9 May 1994)

Summary — Aliphatic ethers **2** and esters **3**, which are closely related to antifungal azoles, were synthesized and tested against various strains of *Candida*. We found that their activity was strongly related to the length of the chains; the best activity was obtained with a C-6 chain for ethers and C-5 or C-6 chains for esters (including the carbonyl group), whereas shorter or longer alkyl chains decreased antifungal efficiency. The biological activity of such compounds could be related to their ability to bind with the lipophilic area near the active site of enzymatic target. The lipophilic character increases with the length of chain, following a linear variation. Thus, even if the activity depends on the lipophilic influence, this particular property is not sufficient to totally explain the antifungal efficiency.

imidazole / antifungal activity / yeast / hydrophobicity / structure–activity relationship

Introduction

Many 1*H*-imidazole derivatives with a broad range of antifungal activity have been synthesized and developed in clinical uses, *eg*, econazole **1a**, miconazole **1b**, and sulconazole **1c** (fig 1). Several aliphatic ethers or esters of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl) ethanol sharing common characteristic features with these compounds and bearing saturated alkyl chains have been presented in patent applications [1–5], but physicochemical and biological data are generally not available. Arylazolylethyl ethers seem to be pharmacophores for antifungal activity [6], even though the antimycotic activity of 1-aryl-2-(1*H*-imidazolyl) ethanols is very poor [7].

The hydrophobicity plays an important role in the structure–activity relationships of compounds of biological interest. The dependence of retention on hydrophobic area and number of carbons in homologous series of compounds follows a linear relationship (for instance, the methylene group shows a constant contribution to retention within a homologous set [8]).

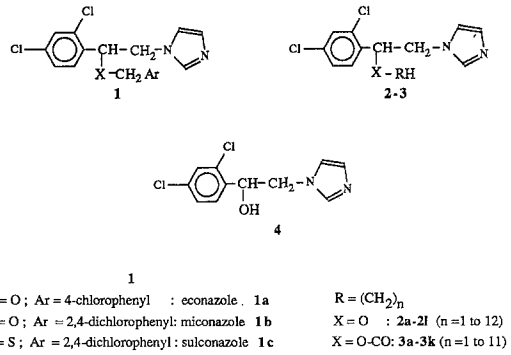
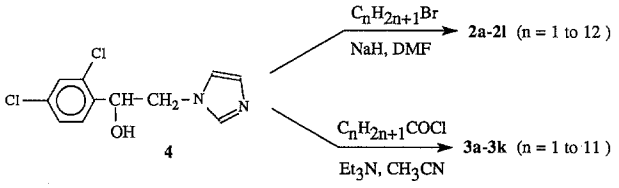


Fig 1. Structure of imidazole derivatives.

The determination of hydrophobic parameters is clearly important to prove the contribution of lipophilic character to the antifungal activity of these compounds. In order to know whether the antifungal activity of imidazole derivatives **2** and **3** was directly reflected by their lipophilic character (described by quantitative parameters like log k_w and R_m), we synthesized 2 sets of compounds and evaluated the influence of chain length on antifungal potency.

*Correspondence and reprints



Scheme 1.

Chemistry

Derivatives **2** and **3** were obtained in a classical manner as described in scheme 1. Physiological data are reported in tables I and II.

Results and discussion

Lipophilic parameters

The lipophilic parameters of the compounds, measured by reverse-phase liquid chromatography and thin-layer chromatography, are reported in the tables III and IV, and compared with those of econazole **1a**, miconazole **1b**, sulconazole **1c** and alcohol **4**. The variations in retention time (R_m) and capacity factors (log k_w) with the length of the alkyl chain are shown in figures 2 (ethers) and 3 (esters).

Antifungal activity

All the compounds and references **1a–c** were tested against an array of clinical isolates: 3 *Candida albicans*; 2 *C glabrata;* 2 *C krusei (Issatchenkia orientalis)*; 2 *C parapsilosis*; and 2 *C tropicalis*.

The results of *in vitro* activities, IC_{90}, of ethers **2a–l** and esters **3a–k**, are reported in table V. When IC_{90} were close for different strains of the same species, we reported a median value. In the reverse case, we gave the range between the smallest and largest IC_{90}. The results of IC_{90} for the ethers derivatives are illustrated in figure 4; the median IC_{90} values were used for these plots.

Antifungal activity is closely related to chain length both for the ethers and esters derivatives. For ethers, $IC_{90} > 100$ µg/ml if $n < 3$ or $n > 9$ and maximum activity occurs for $n = 6$ (**2f**); for esters, $IC_{90} > 100$ µg/ml if $n' < 4$ or $n' > 8$ (n' = carbon number including carbonyl group, $n' = n + 1$) and maximum activity occurs for $n' = 5$ (**3d**) and $n' = 6$ (**3e**). The ethers derivatives are more potent than **3a–k** compounds bearing the same alkyl chain. For ether derivatives, antifungal efficiency is quite heterogeneous, according to the yeast strain and the length of the chain and IC_{90} is between 1 and > 100 µg/ml. It is interesting to note the excellent contribution of derivatives **2f** and **2g** against *C glabrata* ($0.5 < IC_{90} < 4$ µg/ml). This species requires a secondary resistance against oral imidazole drugs, particularly from fluconazole among HIV-positive patients [9]. The efficiencies of derivatives **2h** and **2i** were very different according to the strain.

For the esters derivatives, the IC_{90} are greater than the IC_{90} of ethers but the deviation between the different strains is smaller. As for the ethers, *C glabrata* is the most inhibited strain with *C parapsilosis*. Although antifungal activity exists for *C albicans*, the IC_{90} are greater than the IC_{90} of **1a–c**.

HPLC determination of capacity factors (log k_w) may provide a good approximation of the octanol/water partition coefficient (log P) for a set of closely related compounds [8, 10].

Table I. Physicochemical data of ethers derivatives **2a–i**.

Compound	*n*	*Yield (%)*	$n_D^{23°C}$	R_f*	*Formula*	*Lit*
2a	1	32	1.5508	0.58	$C_{12}H_{12}Cl_2N_2O$	
2b	2	28	1.5450	0.55	$C_{13}H_{14}Cl_2N_2O$	
2c	3	47	1.5408	0.52	$C_{14}H_{16}Cl_2N_2O$	[1]**
2d	4	26	1.5400	0.50	$C_{15}H_{18}Cl_2N_2O$	[1]**
2e	5	18	1.5368	0.47	$C_{16}H_{20}Cl_2N_2O$	
2f	6	28	1.5332	0.43	$C_{17}H_{22}Cl_2N_2O$	
2g	7	28	1.5300	0.38	$C_{18}H_{24}Cl_2N_2O$	
2h	8	17	1.5280	0.32	$C_{19}H_{26}Cl_2N_2O$	
2i	9	40	1.5256	0.29	$C_{20}H_{28}Cl_2N_2O$	
2j	10	25	1.5228	0.27	$C_{21}H_{30}Cl_2N_2O$	
2k	11	20	1.5190	0.23	$C_{22}H_{32}Cl_2N_2O$	
2l	12	13	1.5170	0.20	$C_{23}H_{34}Cl_2N_2O$	

*For determination of R_f, see *Experimental protocols*; **reported and not described.

Table II. Physicochemical data of esters derivatives **3a–k**.

Compound	*n*	*Yield (%)*	$n_D^{23°C}$ *or mp (°C)*	R_f*	*Formula*	*Lit*
3a	1	49	196	0.62	$C_{13}H_{12}Cl_2N_2O_2$	mp = 223°C [3]***
3b	2	83	1.5378	0.62	$C_{14}H_{14}Cl_2N_2O_2$	[3, 4]**
3c	3	74	1.5344	0.59	$C_{15}H_{16}Cl_2N_2O_2$	[3, 4]**
3d	4	71	1.5342	0.58	$C_{16}H_{18}Cl_2N_2O_2$	[5]**
3e	5	97	1.5334	0.56	$C_{17}H_{20}Cl_2N_2O_2$	[5]**
3f	6	68	1.5320	0.53	$C_{18}H_{22}Cl_2N_2O_2$	mp = 124–126°C [5]****
3g	7	95	1.5281	0.48	$C_{19}H_{24}Cl_2N_2O_2$	mp = 99–100.5°C [5]****
3h	8	84	1.5214	0.45	$C_{20}H_{26}Cl_2N_2O_2$	[5]**
3i	9	91	1.5178	0.39	$C_{21}H_{28}Cl_2N_2O_2$	[5]**
3j	10	82	1.5019	0.35	$C_{22}H_{30}Cl_2N_2O_2$	
3k	11	82	1.5014	0.32	$C_{23}H_{32}Cl_2N_2O_2$	mp = 84.5–86.5°C [5]****

*For determination of R_f, see *Experimental protocols*; **reported and not described; ***salt of 1,5-naphthalenedisulfonic acid; ****salt of nitric acid.

Table III. Lipophilic parameters of ethers derivatives **2a–l**, reference compounds **1a–c** and precursor alcohol **4**.

Compound	*n*	R_m *log ((1/R_f)–1)*	*log* k_w*
2a	1	–0.15	2.60
2b	2	–0.09	3.15
2c	3	–0.03	3.74
2d	4	0	4.47
2e	5	0.05	5.04
2f	6	0.12	5.60
2g	7	0.20	6.50
2h	8	0.32	7.09
2i	9	0.39	7.63
2j	10	0.43	8.38
2k	11	0.50	8.86
2l	12	0.59	9.50
1a		0.03	5.07
1b		0.18	5.86
1c		0.05	5.18
4		–0.16	2.72

*See *Experimental protocols* for the determination of log k_w.

Table IV. Lipophilic parameters of esters derivatives **3a–k**.

Compound	*n'* *(n + 1)***	R_m *log ((1/R_f)–1)*	*log* k_w*
3a	2	–0.22	2.75
3b	3	–0.21	3.17
3c	4	–0.16	3.65
3d	5	–0.14	4.03
3e	6	–0.10	4.72
3f	7	–0.05	5.22
3g	8	–0.03	5.74
3h	9	0.09	6.35
3i	10	0.19	6.50
3j	11	0.27	7.05
3k	12	0.32	7.63

*See *Experimental protocols* for the determination of log k_w; **n' = n + 1: carbon number including carbonyl group for esters.

As expected, the R_m and log k_w in tables III and IV show that addition of methylene groups on the aliphatic chain increases compound lipophilicity, following a linear relationship for each set (figs 2 and 3).

Results from antifungal experiments suggest that the ability of these compounds to bind with active sites of enzymatic targets present in yeast (Cyt-P450-dependent enzyme system) is related to the length of the alkyl chain, probably by steric strains. An optimal length, near to 6 carbons (like the benzene ring), can increase binding through the lipophilic area, whereas shorter or longer chains decrease these interactions (fig 4).

Earlier studies on *N*-alkylimidazoles [11, 12] also indicated that a 10-carbon chain was optimal for antibacterial and antifungal activities. For our derivatives, **2a–l** and **3a–k**, the aliphatic chain is linked to imidazole moiety by an X-CH-CH_2 bridge (X = O or OCO). Thus, the total chain length is near to this value.

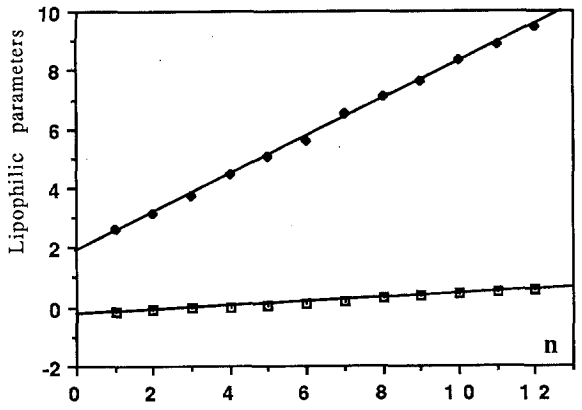


Fig 2. Variations of R_m (⊡) and log k_w (◆) for ethers as a function of the chain length. $R_m = -0.2492 + 0.0682\ n$, $R^2 = 0.988$; log $k_w = 1.8962 + 0.6385\ n$, $R^2 = 0.999$.

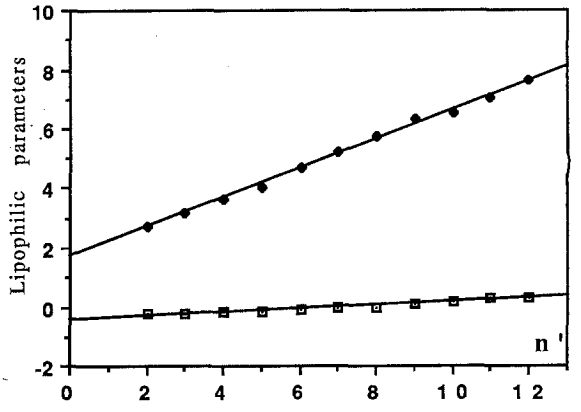


Fig 3. Variations of R_m (⊡) and log k_w (◆) for esters as a function of the chain length. $R_m = -0.3981 + 0.0563\ n'$, $R^2 = 0.943$; log $k_w = 1.7199 + 0.4920\ n'$, $R^2 = 0.996$.

Experimental protocols

Chemistry

General method for preparation of ether derivatives ***2a–l***
1-(2,4-Dichlorophenyl)-2-(1*H*-imidazolyl) ethanol (2.57 g, 0.010 mol) (Jansen) was mixed with 0.7 g (0.014 mol) NaH in DMF. The appropriate alkyl bromide was slowly added over 30 min and the mixture refluxed for 30 min. The solution was concentrated under reduced pressure, and the residue was suspended in water and extracted with methylene chloride. The organic layer was dried on $MgSO_4$, concentrated and purified by chromatography on silica column (eluent: ethyl acetate/methylene chloride 1:4). The formation of secondary products by *N*-alkylation of the imidazoyl heterocycle (on the nitrogen atom in position 3) explains the low yields of ether compounds formation. These by-products are insoluble in the reaction medium, and were eliminated by filtration.

General method for preparation of ester derivatives ***3a–k***
To a solution of 2.57 g (0.010 mol) 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl) ethanol in methylene chloride (20 ml) was added 1.0 g of triethylamine. A convenient dilution of acyl chloride (0.010 mol) in 20 ml methylene chloride was added dropwise and the mixture stirred at room temperature for 4 h.

Table V. IC_{90} of ethers and esters (μg/ml)*.

Compound	*C albicans*	*C glabrata*	*C krusei*	*C parapsilosis*	*C tropicalis*
2c	66.8	81.9–100	75.6	10.8	17.5–100
2d	31.9	21.7–100	26.9	4.3–56.9	18.2
2e	33.8	3.2–42.0	45.0–93.0	6.4	26.6
2f	26.9	1.9	24.7	5.4	16.2
2g	17.6–100	2.7	52–100	9.7	19.6
2h	22.5–100	1.2–34.0	> 100	0.81–14.2	23.6
2i	11.2–100	0.29–100	> 100	0.50–52.4	36.1–100
3c	7.6–43.2	17.2	43.9–100	18.7	28.5–56.9
3d	17.9	11.5	30.6	14.1	16.1
3e	8.9–53.1	14.1	21.7–100	7.2–25.4	18.4–28.3
3f	20.1–100	13.6	19.7–100	15.1	35.1
3g	9.6–100	8.8	> 100	10.9–38.7	29.5–100
1a	8.0–35.4	0.6–7.9	6.2	0.2	0.5
1b	9.5	0.6	4.5	0.5	9.66×10^{-3}
1c	0.4–13.7	8.4	5.1–23.6	0.7	0.6

*Results are not reported for **2a–b**, **2j–l**, **3a–b**, **3h–k** and **4** (IC_{90} > 100 μg/ml).

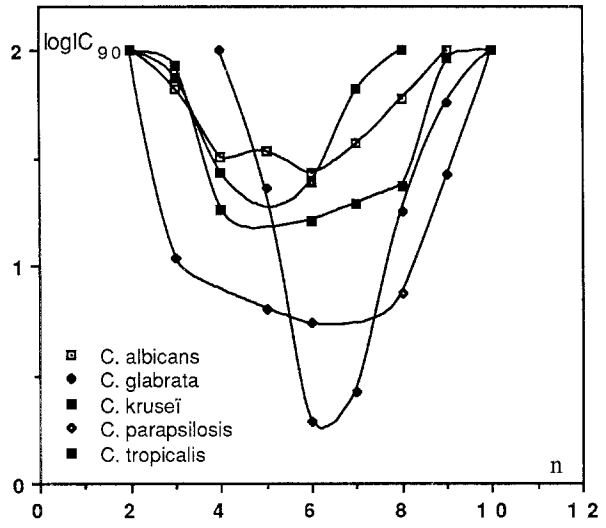


Fig 4. Plots of log IC_{90} variation of ethers as a function of the chain length.

Water (30 ml) was then added and the organic layer evaporated to dryness; the crude product was purified by chromatography on silica column (eluent: ethyl acetate/methylene chloride 1:4).

Physicochemical data for compounds 2 and 3

Physicochemical characteristics and yields of **2** and **3** are given in tables I and II; all the compounds are liquids except **3a**. The melting point of **3a** was determinated on a Kofler bank; refraction values were determined on Abbe refractometer for the other compounds.

Infrared spectrometric data (in KBr pellets or liquid films) were measured with Perkin-Elmer 983G. **2a–l**: (ν in cm^{-1}) 3100: CH imidazole; 2950–2880: CH_3, CH_2, CH; 1600: C=N; 1560: C=C; 1100: C-O. **3a–k**: (ν in cm^{-1}) 3100: CH imidazole; 2940–2860; CH_3, CH_2, CH; 1745: C=O; 1590: C=N; 1560: C=C. NMR data (in $CDCl_3$) at 200 MHz with Me_4Si as an internal standard are reported in tables VI and VII. Elemental analyses are in agreement with the accepted norms and are not reported.

Determination of hydrophobic parameters by reverse-phase liquid chromatography

Retention times were measured at ambient temperature with the flow rate 1 ml/min. The extrapolated capacity factors (log k_w) were calculated as described previously [8, 10].

A chromatograph equipped with an Analprep EC 93 pump was used. The spectrophotometric detector was a Shimadzu SPD 2A model operating at 260 nm. The column (25 cm x 4.6 mm) was prepacked with Kromasil RP-18 (particle size 5 µm). A Shimadzu CR 3A Chromatopac integrator was used for peak registration and calculation of retention times.

The mobile phase was made from combinations of methanol and aqueous sodium acetate (0.05 M) after addition of ammonia to adjust the pH to 9.7. Five combinations containing between 80 and 100% of methanol were made (80, 85, 90, 95 and 100%).

R_f and R_m parameter measurements

The R_f values were measured according to the method described by Bellucci *et al* [13] with silica RP-8 $F_{254}s$ (Merck) plates and methanol/water mixture as solvent (9:1) and then R_m were calculated from R_f.

Antifungal activity

Each isolate was subcultured onto Sabouraud's medium with antibacterials (gentamycine and chloramphenicol). Yeast inocula were diluted in YNBG medium (yeast nitrogen base glucose 2%) to ensure concentration of 10^4 cells/ml. Stock solutions of antifungal agents (100 µg/ml) were made in water/DMSO

Table VI. ^{1}H-NMR of ether derivatives **2a–l** δ (ppm in $CDCl_3$).

Compound	*Alkyl chains* CH_3-$(CH_2)_x$-CH_2-CH_2-O-						*Aromatic ring*			*Imidazole ring*	
	t (or s) CH_3	*m* $(CH_2)_x$	*m* $(CH_2)CH_2O$	*t (or q)* CH_2O	*2dd* CH_2N	*2d* OCH	*d* H_3	*dd* H_5	*d* H_6	*s* H_2	*2s* H_4 *and* H_5
2a	(3.41)	–	–	–	4.09	4.79	7.35	7.10	6.90	7.53	6.93 and 7.01
2b	1.16	–	–	(3.38)	4.14	4.86	7.33	7.21	7.04	7.58	6.94 and 7.01
2c	1.02	–	1.71	3.36	4.07	4.85	7.40	7.22	7.04	7.48	6.93 and 7.00
2d	0.91	1.33	1.51	3.27	4.01	4.82	7.35	7.23	7.05	7.58	6.90 and 7.00
2e	0.89	1.31	1.52	3.32	4.09	4.85	7.39	7.27	7.04	7.47	6.91 and 7.01
2f	0.87	1.28	1.52	3.31	4.13	4.85	7.41	7.25	7.03	7.44	6.92 and 7.01
2g	0.88	1.25	1.53	3.32	4.12	4.80	7.40	7.24	7.04	7.44	6.90 and 7.00
2h	0.88	1.28	1.54	3.35	4.08	4.84	7.39	7.22	7.03	7.45	6.92 and 7.01
2i	0.88	1.25	1.53	3.30	4.03	4.83	7.38	7.21	7.04	7.44	6.92 and 7.01
2j	0.87	1.25	1.53	3.30	4.10	4.82	7.42	7.23	7.05	7.45	6.92 and 7.00
2k	0.88	1.25	1.53	3.30	4.14	4.82	7.41	7.24	7.05	7.45	6.92 and 7.01
2l	0.88	1.25	1.53	3.30	4.14	4.82	7.40	7.23	7.03	7.44	6.92 and 7.01

Table VII. ^{1}H-NMR of ether derivatives **3a–k** δ (ppm in $CDCl_3$).

Compound	*Alkyl chains* CH_3-$(CH_2)_x$-CH_2-CH_2-CO-						*Aromatic ring*			*Imidazole ring*	
	t (or s) CH_3	*m* $(CH_2)_x$	*m* $(CH_2)CH_2CO$	*t (or q)* CH_2O	*2dd* CH_2N	*2d* *OCH*	*d* H_3	*dd* H_5	*d* H_6	*s* H_2	*2s* H_4 *and* H_5
3a	(2.24)	–	–	–	4.81	6.49	7.42	7.25	7.10	7.37	6.84 and 7.01
3b	1.13	–	–	(2.39)	4.32	6.28	7.41	7.20	7.05	7.35	6.80 and 7.00
3c	0.92	–	1.69	2.39	4.32	6.29	7.42	7.20	7.04	7.34	6.88 and 7.00
3d	0.90	1.28	1.59	2.38	4.27	6.26	7.41	7.19	7.05	7.35	6.80 and 7.01
3e	0.87	1.26	1.59	2.36	4.27	6.27	7.38	7.20	7.08	7.33	6.82 and 7.04
3f	0.86	1.26	1.58	2.37	4.27	6.27	7.38	7.21	7.08	7.34	6.81 and 7.04
3g	0.85	1.26	1.59	2.39	4.29	6.28	7.39	7.20	7.09	7.34	6.80 and 7.06
3h	0.86	1.24	1.57	2.36	4.27	6.26	7.36	7.14	7.09	7.33	6.82 and 6.95
3i	0.86	1.24	1.56	2.35	4.28	6.26	7.39	7.13	7.09	7.33	6.81 and 6.94
3j	0.87	1.25	1.59	2.37	4.27	6.28	7.40	7.21	7.06	7.45	6.81 and 7.00
3k	0.87	1.25	1.59	2.37	4.27	6.27	7.40	7.21	7.05	7.41	6.80 and 7.00

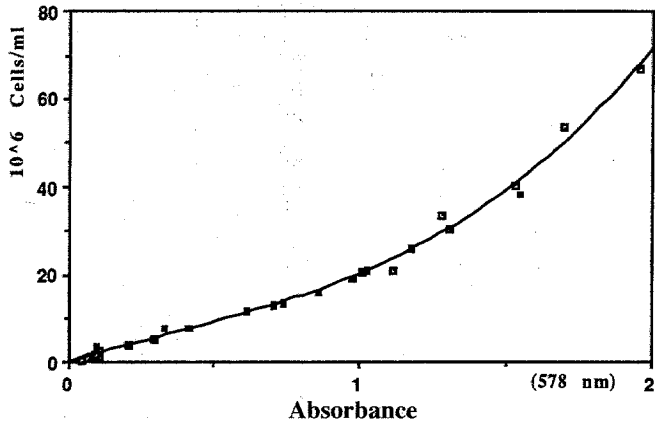


Fig 5. Plot of absorbance at 578 nm as a function of the *C krusei* cell number.

mixture (70:30) with Tween-80 (3.5 g/100 ml); 5 dilutions were obtained with sterile distilled water (50, 25, 10, 2, 1).

IC_{90} determination

IC_{90} values were determined by a turbidimetric method [14] with a Hitachi U1100 spectrophotometer.

In order to relate the number of yeast cells of an inocula with the absorbance measured at 578 nm, the cell number was evaluated by a classical numeration technique (Thomas cell) for each strain. This operation was repeated for a large number of inocula containing different concentrations of yeast. The corresponding absorbance was then measured at 578 nm and plotted as a function of the cell number. Figure 5 gives an example of the plot performed for *C krusei*, which was recently called *Issatchenkai orientalis* [15].

The absorbance was measured for samples composed of 200 µl yeast inocula (to a concentration adjusted to 10^4 cells/ml), added by 100 µl to antifungal solution at different concentrations (100, 50, 25, 10, 2, 1 µg/ml), and after incubation during 24 h at 30°C.

The absorbance was easily converted into the number of cells contained in the sample and the efficiency percentage was calculated from the expression:

$$\% \text{ efficiency} = \frac{\text{No cells in control} - \text{No cells in sample}}{\text{No cells in control}} \times 100$$

The calculated efficiency percentage was converted into the probit given by the Fisher table. The probit was plotted as a function of the antifungal concentration and the IC_{90} could be determined according with the connection between 90% efficiency and a probit of 6.28 indicated by the Fisher table.

References

1 Godefroi EF, Schuerrmans JF (1971) DP 2,063,857 (*Chem Abstr* 75, 118319n)
2 Janssen (1979) British patent 1,522,848 (*Chem Abstr* 91, 175341t)
3 Bayer AG (1978) DP 2628420 (*Chem Abstr* 88, 152617n)
4 Bayer AG (1978) DP 2628421 (*Chem Abstr* 89, 84890p)
5 Syntex Inc (1977) US patent 4,038,409 (*Chem Abstr* 87, 152210c)
6 Ogata M, Matsumoto H, Shimizu S, Kida S, Shiro M, Tawara K (1989) *Eur J Med Chem* 24, 137–143
7 Porretta GC, Fioravanti R, Biava M *et al* (1993) *Eur J Med Chem* 28, 749–760
8 Braumann T (1986) *J Chromatogr* 373, 191–225
9 Hitchcock CA, Pye GW, Troke PF, Cohnson EM, Warnock DW (1993) *Antimicrob Agents & Chemother* 37, 1962–1965
10 El Tayar N, Van De Waterbeemd H, Testa B (1985) *J Chromatogr* 320, 293–304
11 Wilkinson CF, Hetnarski K, Cantwell GP, Dicarlo FJ (1974) *Biochem Pharmacol* 23, 2377–2386
12 De Savignac A, Roques C, Hinedi M, Michel G, Lattes A (1990) *Eur J Med Chem* 25, 449–454
13 Belluci C, Gualtieri F, Chiarini A (1987) *Eur J Med Chem* 22, 473–477
14 Galgiani JN, Stevens DA (1978) *FEBS Lett* 13, 249–254
15 Kwon-Chung KJ, Bennet JE (1992) *Medical Mycology*. Lea and Febiger, Philadelphia, PA 316–318