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Antifungal Activity of Halophenols and Halonitrophenols

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Summary. Thirty one compounds (phenol; its 12 possible monohalo analogues; 18 nitrophenols (2- and 4-nitrophenols, 4-, 5-, and 6-halo-2-nitrophenols, 3-halo-4-nitrophenols)) were tested for antifungal activity against six fungi (*A. niger*, *A. oryzae*, *M. verrucaria*, *T. viride*, *M. cirinelloides*, and *T. mentagrophytes*) in *Sabouraud* dextrose broth. The two most fungitoxic compounds of those studied were 5-fluoro- and 5-iodo-2-nitrophenols which inhibited all the fungi at concentrations under 10 μg/ml. 6-Iodo-2-nitrophenol inhibited five fungi at a concentration below 10 μg/ml and *M. cirinelloides* at 10–100 μg/ml.

Keywords. Antifungal activity; Halophenols; Halonitrophenols.

Fungizide Aktivität von Halogenphenolen und Nitrohalogenphenolen

Zusammenfassung. 31 Verbindungen (Phenol; seine 12 möglichen monohalogenierten Derivate; 18 Nitrophenole (2- und 4-Nitrophenole, 4-, 5- und 6-Halogen-2-nitrophenole, 3-Halogen-4-nitrophenole)) wurden gegenüber 6 Pilzstämmen (*A. niger*, *A. oryzae*, *M. verrucaria*, *T. viride*, *M. cirinelloides*, *T. mentagrophytes*) in *Sabouraud*-Nähmedium auf ihre fungizide Aktivität untersucht. Am effizientesten waren dabei 5-Fluor- und 5-Iod-2-nitrophenole (Hemmung aller Stämme bei Konzentrationen < 10 μg/ml). 6-Iod-2-nitrophenol war gegen 5 Pilze bei Konzentrationen < 10 μg/ml und gegenüber *M. cirinelloides* zwischen 10 und 100 μg/ml aktiv.

Introduction

In the course of our synthetic work [1–3], we routinely screen all intermediates as well as target compounds for antifungal activity. 5-Fluoro-2-nitrophenol was found to possess good toxicity against six fungi: *Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride*, *Mucor cirinelloides*, and *Trichophyton mentagrophytes*.

A search of the literature showed that only a small number of halonitro and halophenols had been tested for antifungal activity, although many were known. 4-Fluoro- and 5-fluoro-2-nitrophenols inhibited some plant pathogenic fungi, among which were *Fusarium sp.* and *Curvularia sp.* at over 200 μg/ml [4]. Moderate inhibitory activity was reported for 2-, 3-, and 4-nitrophenols against *Trichophyton sp.*; 2- and 4-chlorophenols were even less fungitoxic [5]. A variety of fungi were tested against 4-chloro- and 4-bromo-2-nitrophenols. Moderate antifungal activity

was observed [6]. The effect of environmental factors including water hardness, salinity, *pH*, and temperature were studied on the antifungal activity of 2- and 4-chlorophenols against filamentous fungi. Only salinity, comparable to sea water, potentiated toxicity [7]. Twenty five phenols were studied for antifungal activity against *Trichophyton sp.* and *Candida albicans*. Among these compounds were 2-chlorophenol, 2-chloro-4-nitrophenol, 3-chloro-4-nitrophenol, 2-nitrophenol, and its 4-chloro analogue. The most active one was 2-chloro-4-nitrophenol, and there was a significant correlation between activity and pK_a [8]. A comparison of the toxicity of 2- and 4-chloro- and 2- and 4-nitrophenols to dermatophytes showed that the chlorophenols were at least ten times as toxic as the nitrophenols [9]. Moderate fungitoxicity was reported for 2- and 4-chlorophenols against *Fusarium* and *Rhizoctonia sp.* and was related to the undissociated portions of the molecule in the medium [10].

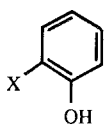
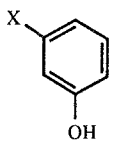
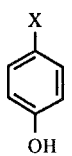
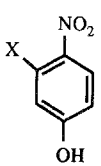
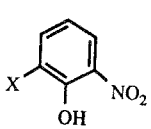
We decided to carry out a more systematic study of the fungitoxicity of phenol and the twelve possible monohalophenols. Also included were 2- and 4-nitrophenols and four sets of halonitrophenols: 4-halo-, 5-halo-, and 6-halo-2-nitrophenols and 3-halo-4-nitrophenols.

Results and Discussion

The minimal inhibiting concentrations (MICs) of all the compounds are summarized in Table 1. We define good activity as inhibition below 10 μg/ml, moderate activity at 10 to 100 μg/ml, and poor activity as between 100 and 1000 μg/ml. The halophenols showed poor antifungal activity against *A. niger*, *A. oryzae*, and *M. cirinelloides* and poor to moderate activity against *M. verrucaria*, *T. viride*, and *T. mentagrophytes*. The fluorinated phenols were poorly active against all the fungi regardless of the position of the fluorine atom on the ring. 2-Chlorophenol was moderately active only against *T. mentagrophytes*, 3-chlorophenol was moderately active against *M. verrucaria*, *T. viride*, and *T. mentagrophytes*. Only *M. verrucaria* and *T. mentagrophytes* were inhibited by 4-chlorophenol at 10 to 100 μg/ml. The bromo and iodo analogues behaved like the chloro analogues.

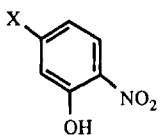
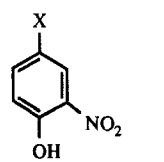
The 3-halo-4-nitrophenols were moderately active against all the fungi with the exceptions of 3-fluoro- and 3-iodo-4-nitrophenols which showed poor activity to *M. cirinelloides*. 6-Halo-2-nitrophenols showed good fungitoxicity against *M. verrucaria* and *T. mentagrophytes* except for the 6-fluoro analogue which was moderately toxic to *M. verrucaria*. 6-Iodo-2-nitrophenol was very toxic to all fungi except *M. cirinelloides* against which it was moderately toxic. The remaining cases of this set of compounds were moderately toxic to all of the fungi. The 5-halo-2-nitrophenols were generally more fungitoxic than the other halonitrophenols in this study. The fluoro and iodo analogues were highly toxic to all six fungi. Good activity was also shown by the chloro and bromo analogues against *A. oryzae*, *M. verrucaria*, *T. viride*, and *T. mentagrophytes*. These two analogues were moderately active against *A. niger* and *M. cirinelloides*. Of the 4-halo-2-nitrophenols, 4-fluoro and 4-bomo-2-nitrophenols were moderately fungitoxic to all six fungi, and 4-chloro-2-nitrophenol was highly toxic to *A. oryzae*, *M. verrucaria*, and *T. mentagrophytes*, whereas 4-iodo-2-nitrophenol showed good activity against *M. verrucaria*, *T. viride*, and *T. mentagrophytes*. These two compounds were moderately toxic to the remaining organisms of the group.

Table 1. Antifungal activity of halophenols and halonitrophenols in *Sabouraud* dextrose broth at 28 °C in shake flasks after six days (μg/ml (mmol/l))

Compound	*X*	Source	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*M. Cirinelloides*	*T. Mentagrophytes*
	H	[11]	>1000 (>10.6)[a]	100 (1.06)	100 (1.06)	100 (1.06)	>1000 (>10.6)	100 (1.06)
	F	[11]	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)
	Cl	[11]	1000 (7.78)	1000 (7.78)	1000 (7.78)	1000 (7.78)	1000 (7.78)	100 (0.78)
	Br	[11]	1000 (5.78)	1000 (5.78)	1000 (5.78)	1000 (5.78)	1000 (5.78)	100 (0.58)
	I	[11]	1000 (4.55)	1000 (4.55)	1000 (4.55)	1000 (4.55)	1000 (4.55)	100 (0.46)
	F	[12]	1000 (8.92)	100 (0.89)	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)
	Cl	[12]	1000 (7.78)	1000 (7.78)	100 (0.78)	100 (0.78)	1000 (7.78)	100 (0.78)
	Br	[12]	1000 (5.78)	1000 (5.78)	100 (0.58)	100 (0.58)	1000 (5.78)	100 (0.58)
	I	[12]	1000 (4.55)	1000 (4.55)	100 (0.46)	100 (0.46)	1000 (4.55)	100 (0.46)
	F	[12]	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)	1000 (8.92)
	Cl	[12]	1000 (7.78)	1000 (7.78)	100 (0.78)	1000 (7.78)	1000 (7.78)	100 (0.78)
	Br	[12]	1000 (5.78)	1000 (5.78)	100 (0.58)	100 (0.58)	1000 (5.78)	100 (0.58)
	I	[12]	1000 (4.55)	1000 (4.55)	100 (0.46)	100 (0.46)	1000 (4.55)	100 (0.46)
	H	[11]	100 (0.72)	100 (0.72)	100 (0.72)	100 (0.72)	1000 (7.16)	100 (0.72)
	F	[13]	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)	1000 (6.34)	100 (0.63)
	Cl	[14]	100 (0.57)	100 (0.57)	100 (0.57)	100 (0.57)	100 (0.57)	100 (0.57)
	Br	[15]	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)
	I	[16]	100 (0.38)	100 (0.38)	100 (0.38)	100 (0.38)	1000 (3.76)	100 (0.38)
	H	[11]	100 (0.72)	100 (0.72)	100 (0.72)	100 (0.72)	1000 (7.16)	100 (0.72)
	F	[17]	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)	6 (0.038)
	Cl	[18]	100 (0.57)	100 (0.57)	5 (0.029)	100 (0.57)	100 (0.57)	3 (0.017)
	Br	[19]	100 (0.46)	100 (0.46)	8 (0.037)	100 (0.46)	100 (0.46)	2. (0.0091)
	I	[20]	7 (0.026)	8 (0.030)	3 (0.011)	7 (0.026)	100 (0.38)	2 (0.0075)

(continued)

Table 1. (*Continued*)

Compound	X	Source	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*M. Cirinelloides*	*T. Mentagrophytes*
	F	[11]	5 (0.032)	3 (0.019)	3 (0.019)	4 (0.025)	4 (0.025)	<1 (<0.0064[b])
	Cl	[14]	100 (0.57)	8 (0.046)	5 (0.029)	6 (0.034)	100 (0.57)	3 (0.017)
	Br	[15]	100 (0.46)	5 (0.023)	4 (0.018)	7 (0.032)	100 (0.46)	3 (0.014)
	I	[16]	10 (0.038)	6 (0.023)	7 (0.026)	8 (0.030)	10 (0.038)	2 (0.0075)
	F	[11]	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)	100 (0.63)
	Cl	[12]	100 (0.57)	5.(0.029)	4 (0.023)	100 (0.57)	100 (0.57)	4 (0.023)
	Br	[21]	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)	100 (0.46)
	I	[22]	100 (0.38)	100 (0.38)	7 (0.026)	9 (0.034)	100 (0.38)	3 (0.011)

[a] The symbol ">" indicates inhibition at >1000 μg/ml (the highest level tested); [b] The symbol "<" indicates inhibition at <1 μg/ml (the lowest level tested)

Thirty one compounds (phenol; its 12 possible monohalo analogues; 18 nitrophenols (2- and 4-nitrophenols, 4-, 5-, and 6-fluoro-, chloro-, bromo-, and iodo-2-nitrophenols, 3-halo-4-nitrophenols)) were tested for antifungal activity against six fungi (*A. niger*, *A. oryzae*, *M. verrucaria*, *T. viride*, *M. cirinelloides*, and *T. mentagrophytes*) in *Sabouraud* dextrose broth. The two most fungitoxic compounds of those studied were 5-fluoro- and 5-iodo-2-nitrophenols which inhibited all the fungi at under 10 μg/ml. 6-Iodo-2-nitrophenol inhibited five fungi at under 10 μg/ml and *M. cirinelloides* at 10–100 μg/ml.

4- and 5-Fluoro-2-nitrophenols were reported to inhibit two plant pathogenic fungi at over 200 μg/ml [4]. The fungi in our test system were inhibited by these compounds at 10 μg/ml and 10–100 μg/ml, respectively. 2- and 4-Chlorophenols were found to be less fungitoxic than the corresponding nitrophenols against *Trichophyton sp.* [5]. Our data did not confirm this. It may be because we did not determine MICs for these compounds. A comparison of the toxicities of 2-chlorophenol with 2-chloro-4-nitrophenol and 4-chlorophenol with 4-chloro-2-nitrophenol against *Trichophyton sp.* indicated that the chloronitrophenols were more toxic than the related chlorophenols. This was related to the pK_as of the compounds [8]. Our data on the same compounds confirmed the fungitoxic action against this species of fungus. That 2- and 4-chlorophenols were reported to be 10 times as toxic to dermatophytes as the 2- and 4-nitrophenols [9] was not confirmed by our data.

Table 2. Proton NMR chemical shifts for halonitrophenols in *DMSO*-d_6 (δ, ppm)

Substituted Phenol	H-2	H-3	H-4	H-5	H-6	O–H
3-fluoro-4-nitro[a]	6.72 (dd)			8.06 (dd)	6.86 (ddd)	11.50 (s)
3-bromo-4-nitro[b]	7.20 (d)			8.00 (d)	6.94 (dd)	11.18 (s)
2-fluoro-6-nitro[c]		7.53 (dq)	7.00 (ddt)	7.74 (dq)		11.39 (s)
2-chloro-6-nitro[d]		7.96 (dq)	7.05 (dd)	7.93 (dq)		11.01 (s)
2-bromo-6-nitro[e]		7.95 (dq)	7.00 (dd)	7.98 (dq)		11.10 (s)
2-iodo-6-nitro[f]		8.00 (dq)	6.87 (dd)	8.14 (dq)		11.15 (s)
5-fluoro-2-nitro[g]		8.05 (dd)	6.76 (dd)		6.95 (dd)	10.80 (s)
5-chloro-2-nitro[h]		7.94 (d)	7.01 (dd)		7.20 (d)	11.40 (s)
5-bromo-2-nitro[i]		7.85 (d)	7.15 (dd)		7.35 (d)	11.20 (s)
5-iodo-2-nitro[j]		7.95 (d)	6.93 (dd)		7.48 (d)	11.02 (s)
4-fluoro-2-nitro[k]		7.77 (dd)		7.47 (ddd)	7.18 (dd)	10.91 (s)
4-chloro-2-nitro[l]		7.93 (d)		7.58 (dd)	7.17 (d)	11.24 (s)
4-bromo-2-nitro[m]		8.04 (d)		7.69 (dd)	7.11 (d)	10.50 (s)
4-iodo-2-nitro[n]		8.13 (d)		7.80 (dd)	6.97 (d)	11.24 (s)
2-bromo-4-nitro[o]		8.14 (dq)		7.69 (dq)	7.11 (dd)	10.93 (s)
2-iodo-4-nitro[p]		7.85 (dq)		7.75 (dq)	6.96 (dd)	10.95 (s)

(s) = singlet; (d) = doublet; (t) = triplet; (q) = quartet; [a] $J_{2,6} = 2.1, J_{5,6} = 9.05$; [b] $J_{2,6} = 2.47, J_{5,6} = 9.05$; [c] $J_{2,3} = 10.76$, $J_{2,4} = 5.00$, $J_{2,5} = 1.63$, $J_{3,4} = 8.24$, $J_{3,5} = 2.19$, $J_{4,5} = 9.05$; [d] $J_{3,4} = 8.23$, $J_{3,5} = 2$, $J_{4,5} = 7.18$; [e] $J_{3,4} = 8$, $J_{3,5} = 2$, $J_{4,5} = 7.18$; [f] $J_{3,4} = 8$, $J_{3,5} = 2$, $J_{4,5} = 7.18$; [g] $J_{3,4} = 9.25$, $J_{3,5} = 6.15$, $J_{4,5} = J_{5,6} = 10.11$, $J_{4,6} = 2.64$; [h] $J_{3,4} = 8.78$, $J_{4,6} = 2.20$; [i] $J_{3,4} = 8.78$, $J_{4,6} = 1.92$; [j] $J_{3,4} = 9.05$, $J_{4,6} = 2.47$; [k] $J_{3,4} = 2$, $J_{3,5} = 9$, $J_{4,5} = 9.05$, $J_{5,6} = 2.47$; [l] $J_{3,5} = 2.70$, $J_{5,6} = 8.79$; [m] $J_{3,5} = 2.20$, $J_{5,6} = 8.80$; [n] $J_{3,5} = 2.20$, $J_{5,6} = 8.79$; [o] $J_{3,5} = 2.2$, $J_{5,6} = 9$; [p] $J_{3,5} = 2.1$, $J_{5,6} = 9.0$ Hz

It seems that the 5-halo-2-nitrophenols manifest their fungitoxicity by means of a nucleophilic reaction against some target. The overall order of reactivity is F > I > Br > Cl. This is consistent with the order of leaving groups. The 3-halo-4-nitrophenols also have activated halogens. These are surprisingly only moderately fungitoxic. It might be that the bulk of the nitro group in the proximity of the halogen interferes with the nucleophilic reaction. The 4- and 6-halo-2-nitrophenols which do not possess activated halogens are generally not highly fungitoxic.

Experimental

The sources of the test compounds are listed in Table 1; the test fungi consisted of *A. niger* (ATCC 1004), *A. oryzae* (ATCC 1101), *M. verrucaria* (ATCC 9095), *T. viride* (ATCC 8678), *M. cirinelloides* (ATCC 7941), and *T. mentagrophytes* (ATCC 9129).

The compounds were tested in *Sabouraud* dextrose broth (Difco Labs, Detroit, MI) according to published methods [23–26]. MICs of the compounds were obtained in μg/ml by serial dilution of dimethyl sulfoxide solutions and recalculated to a molar basis for comparison. Compounds were tested at levels of 1000, 100, and 10 μg/ml. Inhibition was tabulated to the nearest order of magnitude. When toxicity was observed below 10 μg/ml, the MIC was determined to the nearest μg/ml.

^{1}H NMR spectra of the halonitrophenols were taken at 90 MHz on a JEOL FX90Q spectrometer in *DMSO*-d_6 with *TMS* as internal standard. This was done to confirm that the structures shown in Table 1 are correct. Data on chemical shifts and coupling constants are summarized in Table 2.

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