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Evidence of Steric Factors in the Fungitoxic Mechanisms of 8-Quinolinol and Its 2-, 3-, 4-, 5-, 6- and 7-Chloro and Bromo Analogues

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Summary. A study was made of the fungitoxicity of 2-, 3-, 4-, 5-, 6- and 7-chloro and bromo-8-quinolinols against *Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride* and *Trichophyton mentagrophytes* in Sabouraud dextrose broth and in Yeast Nitrogen Base supplemented with 1% *D*-glucose and 0.088% *L*-asparagine. Based on the presence or absence of synergism between pairs of substituted 8-quinolinols and reversal or nonreversal of toxicity by *L*-cysteine or N-acetyl-*L*-cysteine, the following conclusions were reached: (1) substituents on the quinoline ring change the site(s) of action of the toxicant; (2) the sites of action of the 5-, 6-, and 7-chloro-8-quinolinols are different from each other and from 8-quinolinol and its 2-, 3-, and 4-chloro analogues, and the same is true for the corresponding bromo compounds; (3) 8-quinolinol and its 3- and 4-chloro and bromo analogues appear to share common sites of action; (4) for good antifungal activity the 2 position of the ring must not be substituted by sterically bulky groups; (5) the geometry of the binding sites of action are not so constrained that they cannot accommodate the analogously substituted chloro- and bromo-8-quinolinols.

Keywords. Antifungal activity; 2-,3-,4-,5-,6-,7-Chloro and bromo-8-quinolinones; Steric effects; Synergism between isomers; Reversal of toxicity by cysteine and N-acetylcysteine.

Nachweis sterischer Faktoren bei der Fungitoxizität von 8-Chinolinol und seinen 2-, 3-, 4-, 5-, 6- und 7-Chlor- und -Brom-Analogen

Zusammenfassung. Es wurde eine Studie der Fungitoxizität von 2-, 3-, 4-, 5-, 6- und 7-Chlor- und -Brom-8-chinolinol gegenüber *Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride* und *Trichphyton mentagrophytes* in Sabouraud Dextrose Nährmedium und in Hefe-N-Base mit 1% *D*-Glucose und 0.088% *L*-Asparagin unternommen. Auf der Basis des Zutreffens oder der Abwesenheit eines Synergismus zwischen Paaren von substituierten 8-Chinolinolen und der Umkehrung oder Nichtumkehrung der Toxizität durch *L*-Cystein oder N-Acetyl-*L*-cystein wurden folgende Schlußfolgerungen abgeleitet: (1) Substituenten am Chinolin-Ring ändern die Aktionsstelle(n) des Toxikans; (2) Die Angriffsstellen der 5-, 6- und 7-Chlor-8-chinolinole sind untereinander und von 8-Chinolinol und seinen 2-, 3- und 4-Chlor-Analogen verschieden, wobei das auch für die entsprechenden Brom-Verbindungen gilt; (3) 8-Chinolinol und seine 3- und 4-Chlor- und -Brom-Analogen scheinen gemeinsame Aktionsstellen zu teilen; (4) für eine gute antifungale Aktivität darf die 2-Position des Rings nicht mit sterisch anspruchsvollen Gruppen besetzt sein; (5) Die Geometrie des Bindungsstellen der Wirkung ist nicht so gespannt, daß nicht sowohl analoge Chlor- oder Brom-8-chinolinole Platz finden.

Introduction

Antifungal studies were previously reported for mixtures of minimal inhibitory concentrations (MICs) of 8-quinolinol and its 5- and 7-halo analogues. The mixtures of 8-quinolinol and of 5- or 7-fluoro-8-quinolinol and of 5- **and** 7-fluoro-8-quinolinol showed additive activity and their respective toxicities were reversed by *L*-cysteine. These results suggested a common mechanism of action for the three toxicants. Potentiation of fungitoxicity due to mixtures of 8-quinolinol and its 5- and 7-chloro, bromo and iodo analogues, as well as mixtures of 5- **and** 7-chloro-, 5- **and** 7-bromo- and 5- **and** 7-iodo-8-quinolinols was observed. In addition the fungi were not protected from the toxicity of these compounds by *L*-cysteine. This suggested that the modes of action of these compounds are different from each other and from 8-quinolinol and the 5- and 7-fluoro analogues. It was also proposed that the geometry of the 8-quinolinols as influenced by substituents in the 5- and 7-positions of the molecule may determine, in part, their site(s) of fungitoxicity. The studies of synergy were carried out with six fungi *Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride*, *Mucor cirinelloides* and *Trichophyton mentagrophytes* in Sabouraud dextrose broth. The studies of the reversal of toxicity of the compounds was carried out with the same fungi except for *T. mentagrophytes* in Vitamin free Yeast Base supplemented with *L*-asparagine [1].

It was decided to extend this work to include the 2-, 3-, 4- and 6-chloro and bromo-8-quinolinols to learn to what extent steric factors apply to all the monosubstituted chloro and bromo analogues of 8-quinolinol. It was of further interest to determine if mixtures of the pairs of monochloro and monobromo-8-quinolinols substituted in the corresponding positions would exhibit synergism. The five fungi *A. niger*, *A. oryzae*, *M. verrucaria*, *T. viride* and *T. mentagrophytes* in Sabouraud dextrose broth were employed for studies of synergism, and Yeast Nitrogen Base supplemented with *D*-glucose and *L*-asparagine was the medium used to observe the possible reversal of toxicity of the test compounds by *L*-cysteine or N-acetyl-*L*-cysteine against *A. niger*, *A. oryzae*, *M. verrucaria*, and *T. viride*. Yeast Nitrogen Base supplemented with *D*-glucose is similar to Vitamin Free Yeast Base except that Yeast Nitrogen Base contains in addition microgram quantities of B-complex vitamins [2].

Experimental Part

8-Quinolinol, 5-chloro-8-quinolinol, *D*-glucose, *L*-cysteine hydrochloride monohydrate, N-acetyl-*L*-cysteine and *L*-asparagine were purchased from Aldrich Chemical Company, Milwaukee, WI. The remaining 8-quinolinols were prepared according to literature methods: 2-chloro [4], 2-bromo [4], 3-chloro [4], 4-chloro [4], 4-bromo [4], 5-bromo [5], 6-chloro [4], 6-bromo [4], 7-chloro [6] and 7-bromo [6]. The physical constants of the test samples matched those reported.

The test fungi consisted of *A. niger* (ATCC 1004), *A. oryzae* (ATCC 1101), *M. verrucaria* (ATCC 9095), *T. viride* (ATCC 8678), and *T. mentagrophytes* (ATCC 9129). The compounds were tested in Sabouraud dextrose broth (Difco Labs, Detroit, MI) according to published methods [1, 3, 7, 8]. The MICs of the toxicants were obtained in μg/ml by serial dilution of the dimethyl sulfoxide (Me_2SO) solutions and recalculated to a molar basis for comparison. Synergism was sought

by co-dissolving MIC levels of the toxicants in Me_2SO and incorporating them into the growth medium in 10% increments from 10 to 100% of the mixtures. Studies of the reversal of toxicity of the 8-quinolinols by *L*-cysteine or N-acetyl-*L*-cysteine were carried out in Yeast Nitrogen Base (Difco) medium supplemented with 1% *D*-glucose and 0.088% *L*-asparagine. N-Acetyl-*L*-cysteine in Me_2SO solution was used in place of *L*-cysteine hydrochloride in the attempts to reverse the toxicities of 2-chloro and 2-bromo-8-quinolinols against *M. verrucaria* because the required higher concentration of *L*-cysteine hydrochloride reduced the *pH* of the medium significantly, whereas N-acetyl-*L*-cysteine caused insignigicant acidification. Both compounds were shown to be biologically equivalent in these studies.

Results

The MICs of the monochloro- and monobromo-8-quinolinols against the five fungi in Sabouraud dextrose broth and of *A. niger*, *A. oryzae*, *M. verrucaria*, and *T. viride* in the modified Yeast Nitrogen Base are summarized in Tables 1 and 2, respectively. It should be noted that 2-chloro and 2-bromo-8-quinolinols were inactive against all of the fungi except *M. verrucaria* in both media, and that inhibition occurred at a comparatively high levels of compounds. Table 3 contains the data on fungal inhibition in Sabouraud dextrose broth of all the possible binary combinations of the monochloro-8-quinolinols and of the binary mixtures of the monobromo-8-quinolinols. Only the compounds which possessed MIC levels > 1 and $< 100\,\mu g/ml$ were tested. Values above or below these limits were not considered MICs and were not tested. Of the 30 possible mixtures containing MICs of two toxicants against five fungi only 47 cases could be tested. Complete inhibition at 40% or less of the mixture was observed in 39 cases and eight cases showed 100% inhibition at 50% of the level of the mixture. These included 4-chloro **and** 3-chloro-8-quinolinols and 4-bromo- **and** 3-bromo-8-quinolinols against *A. niger*, *A. oryzae*, and *T. viride*, and 2-chloro- **and** 2-bromo-8-quinolinols against *M. verrucaria*.

Table 4 contains the data of the search for synergism between mixtures of the MICs of the pairs of corresponding chloro and bromo-8-quinolinols and between the individual monochloro and monobromo analogues and unsubstituted 8-quinolinol in Sabouraud dextrose broth. Of the 90 possible results 40 could not be tested because the MICs of the individual compounds were < 1 or $> 100\,\mu g/ml$. Fourteen of the mixtures resulted in values of 40% or less of the mixture and were considered synergistic. These include 5-, 6- and 7-chloro- and the corresponding bromo-8-quinolinols mixed with 8-quinolinol. There are also 36 mixtures which showed inhibition at 50% of the mixture and were considered not to be synergistic but rather additive. These include the mixtures of the MICs of the 7-chloro- **and** 7-bromo-8-quinolinols, 6-chloro- **and** 6-bromo-8-quinolinols, 5-chloro- **and** 5-bromo-8-quinolinols, 4-chloro- **and** 4-bromo-8-quinolinols, 3-chloro- **and** 3-bromo-8-quinolinols, and 2-chloro- **and** 2-bromo-8-quinolinols. In addition the mixtures of the MICs of each of 4-chloro-, 3-chloro-, 2-chloro-, 4-bromo-, 3-bromo-, and 2-bromo-8-quinolinols with 8-quinolinol were also additive and not synergistic against any of the same five fungi.

The effect of *L*-cysteine hydrochloride or N-acetyl-*L*-cysteine on the toxicities of the six monochloro, six monobromo and unsubstituted 8-quinolinols in modified

Table 1. Minimal antifungal activity of 8-quinolinol and its six monochloro and six monobromo analogues in Sabouraud dextrose broth at 28 °C in flasks after six days

Compound	Minimal antifungal activity [mmol/l (μg/ml)]				
	A. niger	*A. oryzae*	*M. verrucaria*	*T. viride*	*T. mentagrophytes*
7-Chloro-8-quinolinol[a]	0.017 (3)	0.011 (2)	< 0.0056 (< 1)[b]	0.011 (2)	< 0.0056 (< 1)
7-Bromo-8-quinolinol[a]	0.013 (3)	0.013 (3)	< 0.0045 (< 1)	0.018 (4)	0.0089 (2)
6-Chloro-8-quinolinol	< 0.0056 (< 1)	0.011 (2)	< 0.0056 (< 1)	< 0.0056 (< 1)	< 0.0056 (< 1)
6-Bromo-8-quinolinol	< 0.0045 (< 1)	0.0089 (2)	< 0.0045 (< 1)	< 0.0045 (< 1)	< 0.0045 (< 1)
5-Chloro-8-quinolinol[a]	0.017 (3)	0.011 (2)	< 0.0056 (< 1)	0.017 (3)	< 0.0056 (< 1)
5-Bromo-8-quinolinol[a]	0.013 (3)	0.0089 (2)	< 0.0045 (< 1)	0.013 (3)	< 0.0045 (< 1)
4-Chloro-8-quinolinol	0.022 (4)	0.017 (3)	< 0.0056 (< 1)	0.022 (4)	< 0.0056 (< 1)
4-Bromo-8-quinolinol	0.013 (3)	0.0089 (2)	< 0.0045 (< 1)	0.018 (4)	< 0.0045 (< 1)
3-Chloro-8-quinolinol	0.033 (6)	0.056 (10)	0.022 (4)	0.028 (5)	0.022 (4)
3-Bromo-8-quinolinol	0.040 (9)	0.036 (8)	0.018 (4)	0.040 (9)	0.013 (3)
2-Chloro-8-quinolinol	> 0.56 (> 100)[c]	> 0.56 (> 100)	0.39 (70)	> 0.56 (> 100)	0.28 (50)
2-Bromo-8-quinolinol	> 0.44 (> 100)	> 0.44 (> 100)	0.31 (70)	> 0.44 (> 100)	0.22 (50)
8-Quinolinol[a]	0.14 (20)	0.12 (18)	0.034 (5)	0.14 (20)	0.041 (6)

[a] Data taken from Ref. [1]

[b] The symbol < indicates inhibitory at < 1 μg/ml (the lowest level tested)

[c] The symbol > indicates inhibitory at > 100 μg/ml (the highest level tested)

Table 2. Minimal antifungal activity of 8-quinolinol and its six monochloro and six monobromo analogues in modified yeast nitrogen base[a] at 28 °C in shake flasks after six days

Compound	Minimal antifungal activity [mmol/l (μg/ml)]			
	A. niger	*A. oryzae*	*M. verrucaria*	*T. viride*
7-Chloro-8-quinolinol	0.011 (2)	0.011 (2)	< 0.0056 (< 1)[b]	0.011 (2)
7-Bromo-8-quinolinol	0.0089 (2)	0.013 (3)	0.0089 (2)	0.013 (3)
6-Chloro-8-quinolinol	0.017 (3)	0.011 (2)	< 0.0056 (< 1)	0.022 (4)
6-Bromo-8-quinolinol	0.0089 (2)	0.0089 (2)	< 0.0045 (< 1)	0.018 (4)
5-Chloro-8-quinolinol	0.011 (2)	0.011 (2)	< 0.0056 (< 1)	0.033 (6)
5-Bromo-8-quinolinol	0.013 (3)	0.013 (3)	0.013 (3)	0.026 (6)
4-Chloro-8-quinolinol	0.017 (3)	0.017 (3)	0.011 (2)	0.028 (5)
4-Bromo-8-quinolinol	0.018 (3)	0.013 (3)	0.0089 (2)	0.022 (5)
3-Chloro-8-quinolinol	0.033 (6)	0.033 (6)	0.17 (3)	0.066 (12)
3-Bromo-8-quinolinol	0.031 (7)	0.031 (7)	0.013 (3)	0.052 (12)
2-Chloro-8-quinolinol	> 0.56 (> 100)[c]	> 0.56 (> 100)	0.37 (66)	> 0.56 (> 100)
2-Bromo-8-quinolinol	> 0.44 (> 100)	> 0.44 (> 100)	0.25 (55)	> 0.44 (> 100)
8-Quinolinol	0.055 (8)	< 0.0069 (< 1)	0.014 (2)	0.17 (24)

[a] Medium enriched with 1% *D*-glucose and 0.088% *L*-asparagine

[b] The symbol < indicates inhibitory at < 1 μg/ml (lowest level tested)

[c] The symbol > indicates inhibitory at > 100 μg/ml (the highest level tested)

Table 3. Synergism sought between the combinations of the six monochloro-8-quinolinols and between the corresponding combinations of the six monobromo-8-quinolinols in Sabouraud dextrose broth at 28 °C in shake flasks agter six days

Mixtures of 8-Quinolinols	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*T. mentagrophytes*
7-Chloro + 6-Chloro	NT[a]	30[b]	NT	NT	NT
7-Bromo + 6-Bromo	NT	20	NT	NT	NT
7-Chloro + 5-Chloro[c]	30	30	NT	30	NT
7-Bromo + 5-Bromo[c]	20	30	NT	20	NT
7-Chloro + 4-Chloro	40	30	NT	30	NT
7-Bromo + 4-Bromo	30	30	NT	30	NT
7-Chloro + 3-Chloro	40	30	NT	30	NT
7-Bromo + 3-Bromo	40	30	NT	30	30
7-Chloro + 2-Chloro	NT	NT	NT	NT	NT
7-Bromo + 2-Bromo	NT	NT	NT	NT	NT
6-Chloro + 5-Chloro	NT	30	NT	NT	NT
6-Bromo + 5-Bromo	NT	40	NT	NT	NT
6-Chloro + 4-Chloro	NT	30	NT	NT	NT
6-Bromo + 4-Bromo	NT	30	NT	NT	NT
6-Chloro + 3-Chloro	NT	30	NT	NT	NT
6-Bromo + 3-Bromo	NT	40	NT	NT	NT
6-Chloro + 2-Chloro	NT	NT	NT	NT	NT
6-Bromo + 2-Bromo	NT	NT	NT	NT	NT
5-Chloro + 4-Chloro	40	30	NT	40	NT
5-Bromo + 4-Bromo	30	30	NT	30	NT

Table 3 (continued)

Mixtures of 8-Quinolinols	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*T. mentagrophytes*
5-Chloro + 3-Chloro	40	30	NT	30	NT
5-Bromo + 3-Bromo	40	30	NT	40	NT
5-Chloro + 2-Chloro	NT	NT	NT	NT	NT
5-Bromo + 2-Bromo	NT	NT	NT	NT	NT
4-Chloro + 3-Chloro	50	50	NT	50	NT
4-Bromo + 3-Bromo	50	50	NT	50	NT
4-Chloro + 2-Chloro	NT	NT	NT	NT	NT
4-Bromo + 2-Bromo	NT	NT	NT	NT	NT
3-Chloro + 2-Chloro	NT	NT	50	NT	NT
3-Bromo + 2-Bromo	NT	NT	50	NT	NT

[a] (NT) = not tested because at least one of the components of the mixture inhibits the fungus at < 1 μg/ml (the lowest level tested) or > 100 μg/ml (the highest level tested); these are not MICs

[b] Percent of mixtures containing MICs of each toxicant causing 100% inhibition

[c] Taken from Ref. [1]

Table 4. Search for synergism between mixtures of corresponding chloro- and bromo-8-quinolinols and between 8-quinolinol and the chloro and bromo analogues in Sabouraud dextrose broth at 28 °C in shake flasks after six days

Mixtures of 8-Quinolinols	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*T. mentagrophytes*
7-Chloro + 7-Bromo	50[a]	50	NT[b]	50	NT
6-Chloro + 6-Bromo	NT	50	NT	NT	NT
5-Chloro + 5-Bromo	50	50	NT	50	NT
4-Chloro + 4-Bromo	50	50	NT	50	NT
3-Chloro + 3-Bromo	50	50	50	50	50
2-Chloro + 2-Bromo	NT	NT	50	NT	50
7-Chloro + unsubstituted[c]	20	30	NT	30	NT
6-Chloro + unsubstituted	NT	30	NT	NT	NT
5-Chloro + unsubstituted[c]	30	30	NT	30	NT
4-Chloro + unsubstituted	50	50	NT	50	NT
3-Chloro + unsubstituted	50	50	50	50	50
2-Chloro + unsubstituted	NT	NT	50	NT	50
7-Bromo + unsubstituted[c]	20	40	NT	30	NT
6-Bromo + unsubstituted	NT	30	NT	NT	NT
5-Bromo + unsubstituted[c]	30	30	NT	30	NT
4-Bromo + unsubstituted	50	50	NT	50	NT
3-Bromo + unsubstituted	NT	50	50	50	50
2-Bromo + unsubstituted	NT	NT	50	NT	50

[a] Percent of mixtures containing MICs of each toxicant causing 100% inhibition

[b] (NT) = not tested because at least one of the components of the mixture inhibits the fungus at < 1 µg/ml (the lowest level tested) or > 100 µg/ml (the highest level tested); these are not MICs

[c] Taken from Ref. [1]

Yeast Nitrogen Base against *A. niger*, *A. oryzae*, *M. verrucaria*, and *T. viride* is summarized in Table 5. The results indicate that the protective agents showed no effect against the toxicities of the 5-, 6- and 7-halo-8-quinolinols at 100:1 molar ratios of cysteine to toxicant but did protect against the toxicities of 3- and 4-halo-8-quinolinols as well as 8-quinolinol at 10:1 molar ratios of cysteine to toxicant. The toxicities of 2-chloro- and 2-bromo-8-quinolinols against *M. verrucaria* could not be reversed at the 100:1 molar ratio of N-acetylcysteine to toxicant.

Discussion

In our previous report, we advanced the notion that the geometry of 8-quinolinol as influenced by substituents in the 5- and 7-positions around the ring determines its site(s) of fungitoxicity [1]. Based on the presence or absence of synergism and the protective or nonprotective action of *L*-cysteine against the toxic action of 8-quinolinols, the following conclusions can be drawn: (1) substituents on the quinoline ring can change the site(s) of action of the toxicant; (2) the site(s) of action of the 5-, 6- and 7-chloro-8-quinolinols are different from each other and from 8-quinolinol and its 2-, 3- and 4-chloro analogues, and the same is true for the corresponding

Table 5. Effect of *L*-cysteine on the fungitoxicity of 8-quinolinol and its six monochloro and six monobromo analogues in modified yeast nitrogen base[a] at 28 °C in shake flasks after six days

Compound	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*
7-Chloro-8-quinolinol	–[b]	–	–	–
7-Bromo-8-quinolinol	–	–	–	–
6-Chloro-8-quinolinol	–	–	–	–
6-Bromo-8-quinolinol	–	–	–	–
5-Chloro-8-quinolinol	–	–	–	–
5-Bromo-8-quinolinol	–	–	–	–
4-Chloro-8-quinolinol	+[c]	+	+	+
4-Bromo-8-quinolinol	+	+	+	+
3-Chloro-8-quinolinol	+	+	+	+
3-Bromo-8-quinolinol	+	+	+	+
2-Chloro-8-quinolinol	NT[d]	NT	–[e]	NT
2-Bromo-8-quinolinol	NT	NT	–[e]	NT
8-Quinolinol	+	+	+	+

[a] Medium enriched with 1% *D*-glucose and 0.088% *L*-asparagine

[b] Inhibition not reversed at > 100:1 molar ratio of *L*-cysteine to toxicant

[c] Reversal of inhibited at < 10:1 molar ratio of *L*-cysteine to toxicant

[d] (NT) Not tested because the fungus was not inhibited below 100 µg/ml of toxicant (the highest level tested); these are not MICs

[e] N-acetyl-*L*-cysteine was used as the reversal agent because the 10:1 and 100:1 molar ratios of *L*-cysteine hydrochloride reduced the *pH* of the medium excessively

bromo compounds; (3) 8-quinolinol and its 3- and 4-chloro- and bromo analogues appear to share common sites of action; (4) for good antifungal activity the 2 position of the ring must not be substituted by sterically bulky groups; (5) the geometry of the binding sites of action are not so constrained that they cannot accommodate the analogously substituted chloro and bromo-8-quinolinols.

References and Notes

[1] Gershon H., Clarke D. D., Gershon M. (1991) J. Pharm. Sci. **80**: 542

[2] Modified Yeast Nitrogen Base was used in this study because Vitamin Free Yeast Base was no longer available commercially. These vitamins did not affect the toxicity of 8-quinolinol (3)

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