2001

Antifungal activity of substituted 8-quinolinol-5- and 7-sulfonic acids: a mechanism of action is suggested based on intramolecular synergism / Hermon Gershon, Muriel Gershon, & Donald D. Clarke Harding Laboratory, The New York Botanical Garden, Bronx, New York 10458, USA; Department of Chemistry, Fordham University, Bronx, New York 10458, USA

Herman Gershon
New York Botanical Garden. Harding Laboratory

Muriel Gershon
New York Botanical Garden. Harding Laboratory

Donald Dudley Clarke PhD
Fordham University, clarke@fordham.edu

Mycopathologia **155:** 213–217, 2001.

Antifungal activity of substituted 8-quinolinol-5- and 7-sulfonic acids: a mechanism of action is suggested based on intramolecular synergism

Herman Gershon[1], Muriel Gershon[1] & Donald D. Clarke[2]
[1]*Harding Laboratory, The New York Botanical Garden, Bronx, New York 10458, USA;* [2]*Department of Chemistry, Fordham University, Bronx, New York 10458, USA*

Received 13 February 2001; accepted in final form 23 May 2002

Abstract

8-Quinolinol-5-sulfonic acid was nearly devoid of antimicrobial activity, due to what was believed to be an unfavorable partition coefficient. Since twenty six 8-quinolinol-5- and 7-sulfonic acids were available from our previous work, they were tested against six fungi. The 7-chloro and 7-bromo-5-sulfonic acids and the 5-chloro and 5-bromo-7-sulfonic acids showed fungal inhibition within one order of magnitude of that of 8-quinolinol. It is suggested that a nonchelating mechanism is in part responsible for this fungitoxicity. Five additional 5-sulfonic acids with chlorine in positions 3-, 6-, 3,6-, 3,7-, and 6,7- that were suitable for studies in synergism became available more recently. The enhanced activities of the dichlorosulfonic acids over the correspondingly substituted monochlorosulfonic acids is attributed to intramolecular synergism.

Key words: antifungal activity, halogenated 8-quinolinol-5- and 7-sulfonic acids, halogenated 8-quinolinol-2-methyl-5- and 7-sulfonic acids, intramolecular synergism

Introduction

Early antibacterial studies of 8-quinolinol-5-sulfonic acid showed that the compound was nearly devoid of activity. It was believed that even substituted derivatives would possess no antimicrobial properties. This was rationalized as being due to an unfavourable partition coefficient [1, 2]. During the course of preparing 8-quinolinols, twenty-six 5- and 7-sulfonic acids of 8-quinolinol and its 2-methyl analogue became available. The substituents included fluorine, chlorine, bromine, iodine, nitro and sulfonic acid [3–6]. Additional compounds became available from a recent study which appeared to be suitable for the determination of the presence or absence of intramolecular synergism: 3-chloro, 6-chloro, 3,6-dichloro, 3,7-dichloro, and 6,7-dichloro-8-quinolinol-5-sulfonic acids.

* Published in 2002.

Materials and methods

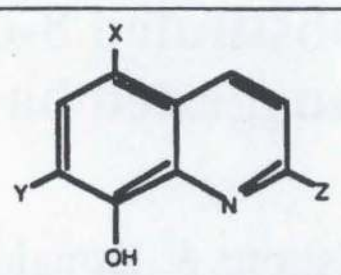
Compounds

8-Quinolinol-5-sulfonic acid and 7-iodo-8-quinolinol-5-sulfonic acids were purchased from Aldrich Chemical Co., Milwaukee, WI. The other 5-sulfonic acids were prepared according to published methods: 7-fluoro [3], 7-chloro [4], 7-bromo [4], 7-nitro [5]. All of the 8-quinolinol-7-sulfonic acids were also reported: 7-fluoro, 7-chloro, 7-bromo, 7-iodo, 7-nitro and 5,7-disulfonic [3]. The corresponding 2-methyl-8-quinolinol-5- and 7-sulfonic acids were reported previously [6]. The 8-quinolinol-5-sulfonic acids with substituents 3-chloro, 6-chloro, 3,6-dichloro, 3,7-dichloro, and 6,7-dichloro have been reported [7], whereas 3,6-dichloro-8-quinolinol-5-sulfonic acid will be reported elsewhere as part of another study.

Antifungal testing

The medium employed was Sabouraud dextrose broth (Difco, Detroit, Michigan), and the test methods were

Table 1. Antifungal activity of 8-quinolinol -5 and -7-sulfonic acids and their respective 7- and 5-fluoro, chloro, bromo, iodo, and nitro analogues in Sabouraud dextrose broth at 28 °C in shake flasks after six days



			Minimal Inhibitory Concentrations [mmol/L (μg/mL)]					
X	Y	Z	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*	*M. cirinelloides*	*T. mentagrophytes*
SO_3H	H	H	>0.44 (>100)[a]	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)
SO_3H	F	H	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)
SO_3H	Cl	H	0.15 (40)	0.077 (20)	0.039 (10)	0.19 (50)	>0.39 (>100)	0.077 (20)
SO_3H	Br	H	0.23 (70)	0.20 (60)	0.066 (20)	0.26 (80)	>0.33 (>100)	0.099 (30)
SO_3H	I	H	>0.28 (>100)	0.20 (70)	0.057 (20)	>0.28 (>100)	>0.28 (>100)	0.11 (40)
SO_3H	NO_2	H	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)
SO_3H	SO_3H	H	>0.33 (>100)	>0.33 (>100)	>0.33 (>100)	>0.33 (>100)	>0.33 (>100)	>0.33 (>100)
H	SO_3H	H	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)	>0.44 (>100)
F	SO_3H	H	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)	>0.41 (>100)
Cl	SO_3H	H	0.15 (40)	0.15 (40)	0.027 (7)	0.23 (60)	>0.39 (>100)	0.077 (20)
Br	SO_3H	H	0.16 (50)	0.13 (40)	0.099 (30)	0.20 (60)	>0.33 (>100)	0.099 (30)
I	SO_3H	H	>0.28 (>100)	>0.28 (>100)	>0.28 (>100)	>0.28 (>100)	>0.28 (>100)	>0.28 (>100)
NO_2	SO_3H	H	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)	>0.37 (>100)
SO_3H	H	CH_3	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)
SO_3H	F	CH_3	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)
SO_3H	Cl	CH_3	>0.37 (>100)	0.29 (80)	0.26 (70)	>0.37 (>100)	>0.37 (>100)	0.18 (50)
SO_3H	Br	CH_3	>0.31 (>100)	0.16 (50)	0.094 ((30)	>0.31 (>100)	>0.31 (>100)	0.063 (20)
SO_3H	I	CH_3	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)
SO_3H	NO_2	CH_3	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)
SO_3H	SO_3H	CH_3	>0.31 (>100)	>0.31 (>100)	>0.31 (>100)	>0.31 (>100)	>0.31 (>100)	>0.31 (>100)
H	SO_3H	CH_3	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)	>0.42 (>100)
F	SO_3H	CH_3	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)	>0.39 (>100)
Cl	SO_3H	CH_3	>0.37 (>100)	0.22 (60)	0.15 (40)	>0.37 (>100)	>0.37 (>100)	0.15 (40)
Br	SO_3H	CH_3	>0.31 (>100)	0.19 (60)	0.094 (30)	>0.31 (>100)	>0.31 (>100)	0.063 (20)
I	SO_3H	CH_3	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)	>0.27 (>100)
NO_2	SO_3H	CH_3	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)	>0.35 (>100)
H	H	H	0.14 (20)	0.12 (18)	0.034 (5)	0.14 (20)	0.17 (24)	0.041 (6)

[a] Test levels: from 10–10^2 μg/mL were carried out in increments of 10 μg/mL and from 1–10 μg/mLin increments of 1 μg/mL.

reported previously. The fungi in the test system included *Aspergillus niger* (ATCC 1004), *A. oryzae* (ATCC 1011), *Myrothecium verrucaria* (ATCC 9095), *Trichoderma viride* (ATCC 8678), *Mucor cirinelloides* (ATCC 7941), and *Trichophyton mentagrophytes* (ATCC 9129) [8–10]. Synergism studies were carried out in Yeast Nitrogen Base (Difco) supplemented with 1% D-glucose (Aldrich) and 0.088% L-asparagine (Aldrich) [9]. All tests were done in duplicate.

Results

The antifungal data of the 26 compounds in Sabouraud Dextrose broth against the six fungi are summarized in Table 1. None of the compounds inhibited *M. cirinelloides*. Of the 8-quinolinol-5- and 7-sulfonic acids, only the 7-chloro and 7-bromo 5-sulfonic acids inhibited five fungi at levels <100 μg/mL, while the 7-iodo analogue inhibited *A. oryzae*, *M. verrucaria*, and *T. mentagrophytes*. The data of the 8-quinolinol-7-sulfonic acids nearly paralleled those of the 5-sulfonic acids, except that 5-iodo-8-quinolinol-7-sulfonic acid

Table 2. Minimal inhibitory concentrations [mmol/L (μg/mL) of some mono and di-chloro-8-quinolinol-5-sulfonic acids in Modified Yeast Nitrogen Base[a] in shake culture after six days at 28 °C

8-Quinolinol-5-sulfonic acids	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*
unsubstituted	0.44 (>100)	0.44 (>100)	0.44 (>100)	0.44 (>100)
3-chloro	0.39 (>100)	0.39 (>100)	0.39 (>100)	0.39 (>100)
6-chloro	0.077 (20)	0.12 (30)	0.077 (20)	0.15 (40)
7-chloro	0.15 (40)	0.12 (30)	0.12 (30)	0.19 (50)
3,6-dichloro	0.010 (3)	0.0068 (2)	0.0068 (2)	0.020 (6)
3,7-dichloro	0.068 (20)	0.068 (20)	0.10 (30)	0.10 (30)
6,7-dichloro	0.014 (4)	0.010 (3)	0.024 (7)	0.068 (20)
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.

Table 3. Determination of synergism between binary mixtures of chlorinated 8-quinolinol-5-sulfonic acids in Modified Yeast Nitrogen Base[a] in shake culture after six days at 28 °C

Mixtures of chlorinated 8-quinolinol-5-sulfonic acids	*A. niger*	*A. oryzae*	*M. verrucaria*	*T. viride*
6-chloro + 7-chloro	0.068 (30)[b]	0.072 (30)	0.080 (40)	0.10 (30)
3,6-dichloro + 7-chloro	0.048 (30)	0.038 (30)	0.038 (40)	0.063 (30)
3,7-dichloro + 6-chloro	0.058 (40)	0.036 (20)	0.071 (40)	0.10 (40)
3,6-dichloro + 6,7-dichloro	0.0072 (30)	0.0034 (20)	0.012 (40)	0.035 (40)
6,7-dichloro + 3,7-dichloro	0.025 (30)	0.023 (30)	0.050 (40)	0.067 (40)
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]Percent of mixtures containing MICs of each toxicant causing 100% inhibition; also expressed as mmol/L for comparison.

did not inhibit any of the fungi. The corresponding chloro and bromo-2-methyl analogues inhibited only *A. oryzae*, *M. verrucaria*, and *T. mentagrophytes* at higher levels of compound than the non-methylated analogues, whereas the remaining 2-methyl analogues were not inhibitory to any of the fungi at <100 μg/mL.

Table 2 contains the minimal inhibitory concentrations (MICs) of 8-quinolinol-5-sulfonic acid and its 3-, 6-, and 7-monochlorinated derivatives along with 3,6-, 3,7-, and 6,7-dichloro-8-quinolinol-5-sulfonic acids in Yeast nitrogen Base. The test fungi included *A. niger*, *A. oryzae*, *M. verrucaria*, and *T. viride*. Synergism data resulting from the monochloro and dichloro-8-quinolinol-5-sulfonic acids against the four fungi in the modified Yeast Nitrogen Base are shown in Table 3. All binary mixtures in the table manifested synergism against the four test organisms. 8-Quinolinol-5-sulfonic acid and its 3-chloro analogue were not included because they did not inhibit the four fungi at <100 μg/mL, the highest level tested (Table 2). 8-Quinolinol was included as a standard antifungal agent for comparison.

Discussion

Comparing the fungitoxicity of the chloro and bromo 8-quinolinol sulfonic acids with 8-quinolinol (Table 1 [11]), it is apparent that these results are within the same order of magnitude for all of the compounds against the five fungi, but only 8-quinolinol inhibited *M. cirinelloides*. Because of the strong acidity of the sulfonic acid substituent, all of the 8-quinolinol sulfonic acids must be in the zwitterionic form and penetrate the fungal cells in the same manner. That the chloro and bromo derivatives are fungitoxic and the related compounds are not, with the exception of 7-iodo-8-quinolinol-5-sulfonic acid (Table 1) suggests a mode of action other than chelation. This had been proposed previously, based on the antifungal data obtained with substituted non-chelating 8-methoxyquinolines [12] and comparably substituted quinolines [13]. This fungitoxicity, although generally weaker, paralleled that of the correspondingly substituted 8-quinolinols.

It was suggested in a prior report that the greater fungitoxicity of 5-, 6-, and 7-haloquinolinols com-

pared to 8-quinolinol was due to intramolecular synergism. This was not true for 8-quinolinols with substituents in positions 2-, 3-, or 4- because these compounds did not form synergistic mixtures between themselves or with 8-quinolinol [14]. 8-Quinolinol-5-sulfonic acid containing chlorine in the 3-, 6-, 7-, 3,6-, 3,7-, and 6,7-positions were available. We undertook the search for synergism of binary mixtures of these compounds in the modified Yeast Nitrogen Base, which is a defined medium.

8-Quinolinol-5-sulfonic acid and its 3-chloro analogue could not be included because they were not fungitoxic <100 μg/mL, and no MICs were determined for them (Table 2). Of the compounds tested in the modified Yeast Nitrogen Base, 3,6-dichloro-8-quinolinol-5-sulfonic acid was the most toxic to all four fungi. It was even more active than 8-quinolinol against all of the fungi except *A. oryzae* for which 8-quinolinol caused 100% inhibition at <1 μg/mL (<0.0069 mmol/L), and the dichlorosulfonic acid caused 100% inhibition of the same organism at 2 μg/mL (0.0068 mmol/L) (Table 2) It should be mentioned that 3,6-dichloro-8-quinolinol was found to be the most fungitoxic of all the 8-quinolinols tested, and the chlorinated 8-quinolinols are more active against the fungi than the correspondingly chlorinated 5-sulfonic acids [7]. This is consistent with the idea that the sulfonic acid substituent imparts an unfavorable partition coefficient to the 8-quinolinol moiety. All of the dichloro-8-quinolinol-5-sulfonic acids were more active than 3,7-dichloro-8-quinolinol-5-sulfonic acid against *M. verrucaria*.

Table 3 contains the antifungal data of binary mixtures of monochloro and dichloro and dichloro-8-quinolinol-5-sulfonic acids. All combinations manifested synergism according to the criterion we employed in that the joint action of the agents when taken together increase each others effectiveness [14–16].

It is fair to say that the enhanced activities of the 6- and 7-chloro sulfonic acids are due to intramolecular synergism, although we were unable to demonstrate it by experiment. Intramolecular synergism was also shown in comparable experiments with binary mixtures 5-, 6-, or 7-halo-8-quinolinols and 8-quinolinol [14]. The compounds with two chlorine substituents on the ring are all more fungitoxic than the monochlorinated sulfonic acids (Table 2). This is similar to the results obtained with the halogenated 8-quinolinols, for which the case of intramolecular synergism was established [7, 14–16].

Where intramolecular synergism can be achieved, it would be an important rational approach to preparing bioactive compounds with improved activity and possible less toxicity. Thus, an agent which simultaneously attacks more than one site in an undesired species would be more difficult to develop resistance to than one which affects only one site. Consequently less xenobiotic material need be added to the environment to achieve a desired result.

References

1. Albert A, Rubbo SD, Goldacre RJ, Balfour BG. The influence of chemical constitution on antibacterial activity. Part III: a study of 8-hydroxyquinoline (oxine) and related compounds. Brit J Exptl Pathol 1947; 28: 69–87.
2. Albert A, Gibson MI, Rubbo SD. The influence of chemical constitution on antibacterial activity. Part IV: the bactericidal action of 8-hydroxyquinoline (oxine). Brit J Exptl Pathol 1953; 34: 119–130.
3. Gershon H, McNeil MW. 8-Quinolinolsulfonic acids. J Org Chem 1971; 36: 3494–3496.
4. Gershon H, McNeil MW, and Grefig AT. Reinvestigation of the action of N-halosuccinimides on bis(8-quinolinolato)copper(II). J Org Chem 1969; 34: 3268–3270.
5. Gershon H, McNeil MW. 7-Iodo-8-quinolinol from 5-amino-8-quinolinol via a Gatterman reaction. J Heterocycl Chem 1971; 8: 129–131.
6. Gershon H, McNeil MW. 5- and 7-Substituted 2-methyl-8-quinolinols. J Heterocycl Chem 1972; 9: 659–667.
7. Gershon H, Clarke DD, Gershon M. Preparation and fungitoxicity of some dichloro-8-quinolinols. Monatsh Chem 1999; 130: 653–659.
8. Gershon H. Antifungal activity of bischelates of 5-, 7-, and 5,7-halogenated 8-quinolinols with copper(II). Determination of approximate dimensions the long and short axes of the pores in the fungal spore wall. J Med Chem 1974; 17: 824–827.
9. Gershon H, Clarke DD, Gershon M. Synergistic antifungal action of 8-quinolinol and its bischelate with copper(II) and with mixed ligand chelates composed of copper(II), 8-quinolinol, and aromatic hydroxy acids. J Pharm Sci 1989; 78: 975–978.
10. Gershon H, Shanks L. Reversal of fungitoxicity of 8-quinolinols and their copper(II) bischelates. Reversal of the Action of 8-quinolinol by DL-α-lipoic acid. Can J Microbiol 1981; 27: 612–615.
11. Gershon H, Clarke DD, Gershon M. Evidence of steric factors in the fungitoxic mechanisms of 8-quinolinol and its 5-halogenated and 7-halogenated analogues. J Pharm Sci 1991; 80: 542–544.
12. Gershon H, Parmegiani R. Secondary mechanisms of antifungal action of substituted 8-quinolinols. I. 5- and 5,7-Substituted 8-methoxyquinolines. Contrib Boyce Thompson Inst 1968; 24: 33–36.
13. Gershon H, Parmegiani R, McNeil MW, Hinds YJ. Secondary mechanisms of antifungal action of substituted 8-quinolinols. II. Substituted quinolines. Contrib Boyce Thompson Inst 1969; 24: 149–150.
14. Gershon H, Gershon M. Intramolecular synergism, an explanation for the enhanced toxicity of halo-8-quinolinols. Monatsh Chem 1995; 126: 1303–1309.

15. The usual definition of synergism is: the joint action of agents when taken together increase each others effectiveness [16]
16. Random House Unabridged Dictionary, 2nd edn. Random House Inc, New York 1993; Grant and Hackh's Chemical Dictionary, 5th. edn. McGraw-Hill, New York (1987).

Address for correspondence: Dr. Herman Gershon, The New York Botanical Garden, Harding Laboratory, Bronx, NY 10458, USA.