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Preparation and Fungitoxicity of 3-Bromo-6-Chloro- and 6-Bromo-3-Chloro-8-Quinolinols

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Summary. 3-Bromo-6-chloro- and 6-bromo-3-chloro-8-nitro, -8-amino-, and -8-hydroxyquinolines along with 3-bromo- and 3-chloroquinolin-6,8-diols were prepared and tested for antifungal activity against six fungi (*Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride*, *Mucor cirinelloides*, *Trichophyton mentagrophytes*) in *Sabouraud* dextrose broth. Compounds with chlorine in the 3 position were generally more fungitoxic than the corresponding analogues with bromine. 6-Bromo-3-chloro-8-quinolinol inhibited four fungi at levels below 1 μg/ml and *A. niger* and *M. cirinelloides* at 2 μg/ml each.

Keywords. 3-Bromo-6-chloro-8-quinolinol; 6-Bromo-3-chloro-8-quinolinol; 3-Bromo-6,8-dihydroxyquinoline; 3-Chloro-6,8-dihydroxyquinoline; Fungitoxicity.

Synthese und Fungitoxizität von 3-Brom-6-chlor- und 6-Brom-3-chlor-8-chinolinolen

Zusammenfassung. 3-Brom-6-chlor- und 6-Brom-3-chlor-8-nitro-, -8-amino- und -8-hydroxychinoline sowie 3-Brom- und 3-Chlorchinolin-6,8-diole wurden hergestellt und gegen sechs Pilzstämme (*Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride*, *Mucor cirinelloides*, *Trichophyton mentagrophytes*) in *Sabouraud*-Dextrosenährmedium auf lhre fungizide Aktivität untersucht. Verbindungen mit Chlor in Position 3 sind durchwegs fungitoxischer als die entsprechenden Bromanalogen. 6-Brom-3-chlor-8-chinolinol hemmt das Wachstum von vier Pilzen bei Konzentrationen unter 1 μg/ml und das von *A. niger* und *M. cirinelloides* bei einer Konzentration von jeweils 2 μg/ml.

Introduction

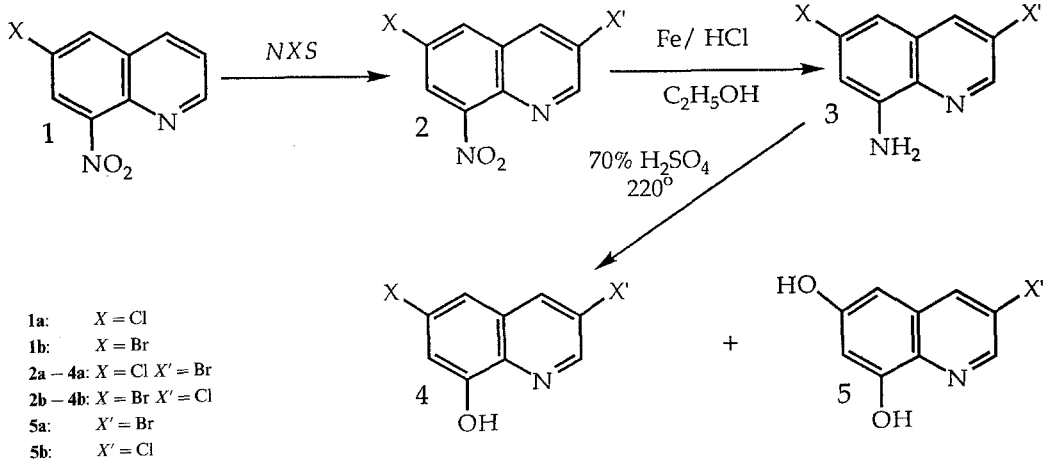
In a previous paper, the preparation and fungitoxicity of 3,6-dichloro- and 3,6-dibromo-8-quinolinols were described [1]. It was reported that the dichloro compound was the most fungitoxic 8-quinolinol of the compounds tested to that time against *Aspergillus niger*, *A. oryzae*, *Myrothecium verrucaria*, *Trichoderma viride*, and *Mucor cirinelloides* in *Sabouraud* dextrose broth. The dibromo analogue was not quite as active. To determine the extent to which the 3 and 6 substituents influence one another with regard to antifungal activity, the preparation of 3-bromo-6-chloro- and 6-bromo-3-chloro-8-quinolinols was undertaken.

In earlier studies on 8-quinolinol and its monohalo analogues, based on the presence or absence of synergism between pairs of compounds and reversal or non-reversal of toxicity of the compounds by *L*-cysteine, the following generalizations were made:

(1) substituents placed on the quinoline ring can alter the sites of action of the compound;
(2) 5-, 6-, and 7-chloro-8-quinolinols act at different sites from each other and from 8-quinolinol and its 2-, 3-, and 4-chloro analogues; the bromo compounds behave similarly;
(3) 8-quinolinol and its 3- and 4-chloro and bromo analogues seem to act at the same sites;
(4) the geometries of the binding sites of action are not so constrained that they can accomodate analogously substituted chloro- and bromo-8-quinolinols [2].

Results and Discussion

3-Bromo-6-chloro- and 6-bromo-3-chloro-8-quinolinol (**4a**, **4b**) were prepared by halogenating 6-chloro- and 6-bromo-8-nitroquinoline (**1a**, **1b**) [1] in acetic acid with N-chloro- or N-bromosuccinimide (*NXS*). The nitro compounds were reduced to amino derivatives (**3a**, **3b**) using Fe/HCl in 95% ethanol. The 8-quinolinols (**4a**, **4b**) were obtained by hydrolysis of the amino compounds in 70% aqueous sulfuric acid (w/w) at 220 °C in a sealed vessel. As a result of this hydrolysis, significant quantities of 3-halo-6,8-dihydroxyquinolines (**5a**, **5b**) were recovered. Hydrolysis of the 6-halo substituent was not noticed as a byproduct of the hydrolysis of 8-amino-6-haloquinolines or of 8-amino-3,6-dihaloquinolines where both halogens were the same [1]. The synthetic procedure described here is shown in Scheme 1.



Scheme 1

In earlier studies, incorrect structures were reported for some halogen substituted 8-quinolinols due to *Reverdin* rearrangements [3]. These were later corrected with the aid of ^{1}H NMR [4]. To avoid this type of error, not only ^{1}H but also

Table 1. ^{13}C NMR Spectra of monochloro- and monobromo-8-quinolinols[a]; chemical shifts (δ) in ppm from *TMS*; carbon atoms bearing a halo substituent are underlined

Substituent	Carbon No.								
	2	3	4	5	6	7	8	4a	8a
2-Chloro	148.2	122.7	140.0	118.1	128.1	113.6	152.9	128.1	138.3
3-Chloro	146.9	128.2	134.3	117.4	129.2	112.4	153.8	129.5	136.9
4-Chloro	147.3	121.8	141.7	113.1	129.0	112.8	153.4	126.4	138.5
5-Chloro	149.0	123.1	132.5	118.7	127.7	111.7	153.2	126.2	139.2
6-Chloro	148.7	123.1	135.6	116.6	131.8	112.2	155.0	129.4	137.6
7-Chloro	149.2	122.2	136.5	118.4	128.5	116.1	149.5	127.5	139.0
2-Bromo	139.2	125.8	139.1	117.8	127.9	113.2	152.6	127.8	138.7
3-Bromo	148.4	117.08	139.2	117.05	128.9	112.3	153.5	129.9	136.6
4-Bromo	147.6	125.6	132.8	115.7	129.0	112.4	153.6	127.8	138.9
5-Bromo	148.7	123.1	134.7	108.2	130.8	112.1	153.4	127.1	139.1
6-Bromo	148.8	123.0	135.5	119.9	120.2	114.8	154.9	130.0	137.7
7-Bromo	148.7	122.0	136.2	118.5	130.5	105.1	150.5	127.6	138.4

[a] Spectra taken in *DMSO*-d_6 with center peak of *DMSO* set at 39.7 ppm from *TMS*

^{13}C NMR spectra of the compounds which might be expected to undergo such rearrangements were recorded. Included were the halogenated 8-nitro compounds and the haloquinolinols resulting from the acid hydrolysis of the 8-amino derivatives. ^{13}C NMR spectra can be used to distinguish easily between carbon bound to chlorine or to bromine at the various positions in question, since these halogens cause shifts of some 6 ppm in opposite directions from the parent C–H compounds. To calibrate the positions of expected chemical shifts more precisely, the ^{13}C NMR spectra the previously available monohalogenated 8-quinolinols [5] were measured (Table 1).

The rates of halogenation of position 3 of the 8-nitroquinolines were much greater than those of position 6, and no undesired products resulted. Eight new compounds (**2–5 a, b**) were produced and were characterized by ^{1}H NMR spectra (Table 2) and ^{13}C NMR spectra (Table 3).

The compounds were tested for antifungal activity in *Sabouraud* dextrose broth (Difco) according to published methods [6–8]. Six fungi were employed (*Aspergillus niger* (ATCC 1004), *A. oryzae* (ATCC 1101), *Myrothecium verrucaria* (ATCC 9095), *Trichoderma viride* (ATCC 8678), *Mucor cirinelloides* (ATCC 7941), *Trichophyton mentagrophytes* (ATCC 9129)). The results are listed in Table 4 and are reported as minimal inhibitory concentrations (MICs) in μg/ml and mmol/l for comparison.

It is apparent that among the 3,6-dihalo-8-nitroquinolines, 6-bromo-3-chloro-8-nitroquinoline (**2b**) possesses significant antifungal activity, whereas the reverse isomer (**2a**) is almost devoid of fungitoxicity. 8-Amino-6-bromo-3-chloroquinoline (**3b**) is also somewhat more active than 8-amino-3-bromo-6-chloroquinoline (**3a**). A clear distinction as to superior antifungal activity cannot be made between 3-bromo- and 3-chloro-6,8-dihydroxyquinolines (**5a, 5b**). The 3,6-dihalo-8-quinolinols (**4a, 4b**) are completely inhibitory to four fungi below 1 μg/ml, the lowest level tested. Against *A. niger* and *M. cirinelloides*, where inhibition was at levels above 1 μg/ml, **4b** was distinctly more toxic than **4a**. From these results it appears

Table 2. ^{1}H NMR Spectra of 3-bromo-6-chloro- and 6-bromo-3-chloro-8-nitro-, -8-amino, and -8-hydroxyquinolines and 3-bromo- and 3-chloro-6,8-dihydroxyquinolines[a]; chemical shifts (δ) in ppm from *TMS*

	Proton No.					Coupling Constant (Hz)	
	2	4	5	7	O or N	J_{24}	J_{57}
3-Br-6-Cl-8-NO_2 **2a**	9.10(d)	8.85(d)	8.53(d)	8.36(d)		1.92	2.20
6-Br-3-Cl-8-NO_2 **2b**	9.05(d)	8.70(d)	8.60(d)	8.54(d)		2.20	1.92
8-NH_2-3-Br-6-Cl **3a**	8.73(d)	8.48(d)	7.07(d)	6.86(d)	6.37(s)	[b]	[b]
8-NH_2-6-Br-3-Cl **3b**	8.69(d)	8.31(d)	7.23(d)	6.99(d)	6.34(s)	[b]	[b]
3-Br-6-Cl-8-OH **4a**	8.87(d)	8.61(d)	7.50(d)	7.14(d)	10.69(s)	2.20	1.92
6-Br-3-Cl-8-OH **4b**	8.82(d)	8.44(d)	7.63(d)	7.23(d)	10.69(s)	2.20	1.92
3-Br-6,8-diOH **5a**	8.60(d)	8.40(d)	6.74(d)	6.65(d)	10.06(s)	2.19	2.47
3-Cl-6,8-diOH **5b**	8.51(d)	8.24(d)	6.68(d)	6.62(d)	10.02(s)	2.20	2.47

[a] Spectra taken in *DMSO*-d_6 with *TMS* as internal standard; [b] these signals are too poorly resolved to determine the coupling constants

Table 3. ^{13}C NMR Spectra of 3-bromo-6-chloro- and 6-bromo-3-chloro-8-nitro-, -8-amino-, and -8-hydroxyquinolines, and 3-bromo- and 3-chloro-6,8-dihydroxyquinolines[a]; chemical shifts (δ) in ppm from *TMS*

	Carbon number								
	2	3	4	5	6	7	8	4a	8a
3-Br-6-Cl-8-NO_2 **2a**	153.6	119.7	137.3	129.9	131.0	124.3	148.2	130.1	135.3
6-Br-3-Cl-8-NO_2 **2b**	152.0	130.0	134.0	133.4	119.2	126.6	148.2	130.5	137.2
8-NO_2	152.6	123.2	136.5	132.0	125.8	123.2	147.9	128.5	136.5
3-Br-6-Cl-8-NH_2 **3a**	147.5	118.0	136.4	111.0	133.5	108.8	147.4	130.2	134.1
6-Br-3-Cl-8-NH_2 **3b**	145.7	128.7	132.9	114.1	122.4	111.2	146.9	129.9	134.1
8-NH_2	146.9	121.3	135.7	113.9	127.5	108.9	145.1	128.6	137.7
3-Br-6-Cl-8-OH **4a**	148.9	118.4	136.6	115.8	133.0	112.8	154.5	130.3	135.3
6-Br-3-Cl-8-OH **4b**	147.1	129.0	133.2	119.0	121.5	115.3	154.8	130.1	135.8
8-OH	148.1	121.8	136.0	117.8	127.5	111.2	153.3	128.9	138.6
3-Br-6,8-diOH **5a**	144.8	117.7	135.3	104.5	157.9	99.0	154.8	131.3	132.7
3-Cl-6,8-diOH **5b**	143.0	128.4	132.1	104.3	157.9	99.0	154.6	130.6	132.7

[a] Spectra taken in *DMSO*-d_6; center peak of *DMSO*-d_6 taken as a secondary standard at 39.7 ppm from *TMS*

that chlorine in position 3 of these types of compounds yields products with superior activity to analogues with bromine in that position.

Mixtures of the MICs of **4a** and **4b** were not synergistic against *A. niger* and *M. cirinelloides* (data not shown). This is consistent with the earlier observation that the geometries of the binding sites are not so constrained that they are able to bind analogously substituted chloro- and bromo-8-quinolinols [2].

Table 4. Minimal antifungal activity (mmol/l (μg/ml)) of 3-bromo-6-chloro- and 6-bromo-3-chloro-8-nitroquinolines, -8-aminoquinolines, and -8-quinolinols, and 3-bromo- and 3-chloro-6,8-dihydroxyquinolines in *Sabouraud* dextrose broth at 28 °C in shake culture after six days[a]

		A. niger	*A. oryzae*	*M. verrucaria*	*T. viride*	*M. cirinelloides*	*T. mentagrophytes*
3-bromo-6-chloro-8-nitro	**2a**	>3.5 (10^3)	>3.5 ($>10^3$)	>3.5 ($>10^3$)	>3.5 ($>10^3$)	>3.5 ($>10^3$)	3.5 (10^3)
6-bromo-3-chloro-8-nitro	**2b**	3.5 (10^3)	0.028 (8)	0.024 (7)	3.5 (10^3)	3.5 (10^3)	0.021 (6)
8-Amino-3-bromo-6-chloro	**3a**	>3.9 ($>10^3$)	>3.9 ($>10^3$)	>3.9 ($>10^3$)	>3.9 ($>10^3$)	>3.9 ($>10^3$)	3.9 (10^3)
8-Amino-6-bromo-3-chloro	**3b**	>3.9 ($>10^3$)	3.9 (10^3)	3.9 (10^3)	>3.9 ($>10^3$)	>3.9 ($>10^3$)	3.9 (10^3)
3-bromo-6-chloro-8-hydroxy	**4a**	0.015 (4)	<0.0039 (<1)	<0.0039 (<1)	<0.0039 (<1)	3.9 (10^3)	<0.0039 (<1)
6-bromo-3-chloro-8-hydroxy	**4b**	0.0077 (2)	<0.0039 (<1)	<0.0039 (<1)	<0.0039 (<1)	0.0077 (2)	<0.0039 (<1)
3-bromo-6,8-dihydroxy	**5a**	0.42 (10^2)	0.25 (60)	0.13 (30)	0.42 (10^2)	4.2 (10^3)	0.083 (20)
3-chloro-6,8-dihydroxy	**4b**	0.46 (90)	0.21 (40)	0.15 (30)	0.25 (50)	5.1 (10^3)	0.15 (30)

[a] Test levels: 10, 10^2, and 10^3 μg/ml; levels from 10 to 10^2 μg/ml were carried out in increments of 10 and from 1 to 10 increments of 1 μg/ml

Previously reported results [1] showed that 3-chloro-8-quinolinol was more fungitoxic than the bromo analogue, and as mentioned earlier, 3,6-dichloro-8-quinolinol was more active than the 3,6-dibromo analogue. The pairs of 8-amino bromo and chloro analogues (**3a, b**) were generally not distinguishable from one another. These results are generally consistent with those of the present report. The major contribution to the fungitoxic activity of the dihalogenated quinolinols is due to the chlorine and bromine substituents in the 6 position. Under these test conditions they are indistinguishable [2]. Chlorine in position 3 of 8-quinolinol causes greater enhancement of fungitoxicity than bromine in the same position. 3,6-Dichloro-8-quinolinol inhibited all test fungi below 1 μg/ml except *M. cirinelloides* which required 7 μg/ml for MIC. Compound **4b** inhibited all test fungi at MICs under 1 μg/ml except *A. niger* and *M. cirinelloides*. The MICs for these organisms were 2 μg/ml each.

Experimental

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. The purity of samples was established by ^1H and ^{13}C NMR spectroscopy at 90 MHz and 22.5 MHz with a JEOL JNM-FX90Q spectrometer using *DMSO*-d_6 as solvent and *TMS* as internal standard. Reactions were monitored by gas chromatography using a Varian Aerograph Model 1400 gas chromatograph with a flame ionization detector to which a Varian Model 20 recorder was attached. The column employed was 5 feet × 1/8 inch o.d., packed with 10% SE-30 on ChromosorbW, and nitrogen was used as the carrier gas. Elemental analyses matched the calculated values satisfactorily for **2–4 a, b** (C, H, N) and for **5a, 5b** (C, H, Br or Cl, N).

3-Bromo-6-chloro-8-nitroquinoline (**2a**; $C_9H_4BrClN_2O_2$)

To a solution of **1a** [1] (14.6 g, 0.07 mol) in 250 ml of acetic acid at 100 °C *NBS* (13.2 g, 0.074 mol) was added in small portions over 1 h with stirring. The solution was heated to boiling, and stirring was continued for 2 h without further heating. The reaction mixture was monitored by gas chromatography for the disappearance of starting materials. The mixture was poured into 800 ml of water, stirred for 1/2 h, and filtered to recover the product. After washing with water and air drying, the crude material weighed 20 g (99%), m.p.: 179–180 °C. The analytical sample obtained by crystallization from a mixture of 95% ethanol and acetonitrile and decolorizing with carbon (Darco G-60) had a m.p. of 183–184 °C.

6-Bromo-3-chloro-8-nitroquinoline (**2b**; $C_9H_4BrClN_2O_2$)

The title compound was prepared from **1b** [1] and *NCS* in acetic acid in the same manner as **2a**. The yield from a 0.05 mol batch was 14.4 g (99.8%), m.p.: 156–157 °C. An analytical sample was obtained from 95% ethanol; m.p. 165–166 °C.

8-Amino-3-bromo-6-chloroquinoline (**3a**; $C_9H_6BrClN_2$)

A mixture of **2a** (20.3 g, 0.071 mol), iron powder (16.8 g, 0.29 g atom), 500 ml of 95% ethanol, and concentrated hydrochloric acid (1.6 ml) was heated under reflux for 3 h with stirring. After removal of the excess iron and its oxides by filtration, the filtrate was poured into 2500 ml of water and stirred for 10 min. The product was obtained by filtration, washed with water, and air dried. The yield of compound **3a** was 17.4 g (95%), m.p.: 117–118 °C. An analytical sample was crystallized from 95% ethanol with decolorization with Darco G-60; m.p.: 124–125 °C.

8-Amino-6-bromo-3-chloroquinoline (**3b**; $C_9H_6BrClN_2$)

Compound **2b** was reduced to the title compound in the same manner as **3a** was obtained from its precursor nitro compound. The product from a 0.04 mol batch was 10 g (97%), m.p.: 118–119 °C. An analytical sample was crystallized from 95% ethanol with Darco G-60 decolorization; m.p.: 126–127 °C.

3-Bromo-6-chloro-8-quinolinol (**4a**; $C_9H_5BrClNO$)

A mixture of **3a** (7.8 g 0.03 mol), 31 ml of water, and 21.2 ml of sulfuric acid (10.3 g) in a glass tube was sealed in a stainless steel pressure vessel containing a small amount of water. The vessel was kept at 220 °C for 8 h, after which it was cooled to room temperature. The hydrolyzate was transferred to 500 ml of water and adjusted to *pH* 7 with ammonium hydroxide. The precipitate was removed by filtration, washed with water, and air dried. The crude material which weighed 7.7 g was extracted with hot acetone and yielded a residue of 6 g of product after evaporation of the solvent. This was composed of two products as determined by ^1H NMR spectroscopy. The title compound was separated from the mixture by steam distillation, filtration, and air drying in 3.5 g (45%) yield, m.p.: 167–172 °C. An analytical sample was crystallized from aqueous ethanol followed by a second crystallization from acetonitrile; m.p.: 176–177 °C.

3-Bromo-6,8-dihydroxyquinoline (**5a**; $C_9H_6BrNO_2 \cdot H_2O$)

The hot aqueous residue from the steam distillation was treated with Darco G-60 and filtered to remove some tarry material. The filtrate was kept under a stream of air to both cool and concentrate it. A yellow orange crystalline product formed which was recovered by filtration and air dried. The yield of comound **5a** was 2.1 g (29%), m.p.: 177–180 °C. A second crystallization from water in the presence of Darco G-60 sharpened the m.p. to 179–180 °C.

6-Bromo-3-chloro-8-quinolinol (**4b**; $C_9H_5BrClNO$)

The title compound was prepared by hydrolysis of **3b** in the same manner as for the preparation of **4a** from its corresponding amino analogue. A 0.03 mol batch of starting compound yielded 2.8 g (36%) of product after steam distillation, m.p.: 157–160 °C. An analytical sample was crystallized from acetonitrile; m.p.: 161–162 °C.

3-Chloro-6,8-dihydroxyquinoline (**5b**; $C_9H_6ClNO_2$)

The hot aqueous residue after steam distillation and treatment with Darco G-60 yielded 2.0 g (34%) of the title compound, m.p.: 178–180 °C. An analytical sample was crystallized from water with Darco G-60 decolorization; m.p.: 182–184 °C.

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