Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 24, pp. 3153-3158, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10176-7

1,4-BIS-(2,3-EPOXYPROPYLAMINO)-9,10-ANTHRACENEDIONE AND RELATED COMPOUNDS AS POTENT ANTIFUNGAL AND ANTIMICROBIAL AGENTS

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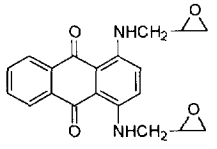
Abstract: 1,4-Bis-(2,3-epoxypropylamino)-9,10-anthracenedione (**1**) and related derivatives were assayed for antifungal, antimicrobial, and plant growth regulatory activity. Compound **1** was extremely potent against several strains of fungi, bacteria, and algae (MIC <2 ppm) and controlled two types of downy mildew by 90–100% at a dose of 12 ppm. © 1997 Elsevier Science Ltd.

We previously reported the synthesis and antitumor activity of the lead compound 1,4-bis-(2,3-epoxypropylamino)-9,10-anthracenedione (**1**) and related derivatives with anthraquinone, napthoquinone, and quinone skeletons.[1] The structural modifications included replacing the amino linkage with an ether linkage to generate 1,4-bis-(2,3-epoxypropoxy)-9,10-anthracenedione (**2**). Previous to this publication, these and related compounds were documented as dyes (**1**),[2] as intermediates in the preparation of drugs (**2**), and as polymer crosslinking agents (**2**).[3] Herein, we report the antifungal and antimicrobial activity and plant growth regulatory properties of **1** and **2** and of related compounds.[4]

Compound **1** was assayed for in vitro growth inhibition against several types of fungi, bacteria, and algae. The results are shown in Tables 1–5. Significant activity (minimum inhibitory concentration, MIC <1 ppm) was found against *Phytophthora capsici*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Aspergillus niger*, *Aureobasidium pullulans*, *Cladosporium resinae*, and *Chaetomium globosum*. Compound **2** was less potent showing microbicidal activity (MIC = 4 ppm) only against *Pseudomonas fluorescens* when tested against six

strains of fungi and bacteria. Likewise, **1** was more potent than **2** against *Pythium ultimum*; the EC_{50} value of the former was 0.03 ppm, while that of the latter was 3.6 ppm. Overall, **1** had similar antimicrobial activity compared with standard commercial biocides. In vivo fungicidal tests using cucumber and grape downy mildew showed that **1** controlled the disease by 90–100% at a dose of 12 ppm. Compound **1** also demonstrated significant plant growth regulation activity in a tobacco root assay. The EC_{50} value was 0.22 ppm compared with values of 0.95 and 0.45 ppm for the known agents Treflan® and chlorpropham.

In a basic medium, bis-3-halo-2-hydroxypropylamino substituted anthracenediones could generate in situ the corresponding epoxy compound through displacement of the halide by the hydroxy group. Accordingly, the 3-chloro-2-hydroxy (**3**) and 3-bromo-2-hydroxy (**4**) derivatives of **1**, along with **1** itself, were tested against *Pseudomonas fluorescens* at different pH values. Table 5 contains the results. Compound **1** was significantly active (MIC <1 ppm) at each pH, while the corresponding bromo compound **4** showed a marked pH dependence with MIC values of 100 ppm at slightly acidic pH (5.5), 25 ppm at neutral pH (7.0), and 0.2 ppm at a basic pH (9.0). In contrast, the chloro compound **3** was relatively inactive at each pH.



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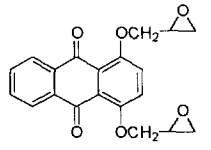

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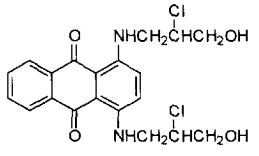

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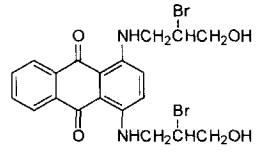

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In summary, 1,4-bis-(2,3-epoxypropylamino)-9,10-anthracendione (**1**) and 1,4-bis-(2,3-epoxypropoxy)-9,10-anthracenedione (**2**) show significant fungicidal and microbicidal activity. These lead structures represent a new class of antifungal and antimicrobial agents with possible uses as disinfectants, preservatives, agricultural fungicides, and sanitizers. Further synthetic and biological studies are in progress to further elucidate the potential of this compound class.

Table 1. Activity of Anthraquinones **1** and **2** at pH 7.5 *vs*. Fungi

Organism	Minimum Inhibitory Concentration, MIC (ppm)		
	1	**2**	Standard[a]
A. niger	0.4, 0.25*	>250	1.6, 0.25*
A. pullulans	0.8, 2.5*	>250	0.1, <0.25*
C. resinae	0.4, <0.25*	NT	25, 2.5*
C. globosum	0.4, <0.25*	NT	1.6, 2.5*
P. funiculosum	0.8, 2.5*	NT	0.8, 2.5*
P. rubra	>25*	NT	2.5*

[a]Iodopropynyl N-*n*-butyl carbamate, a commercial biocide
*Separate test: endpoint 0.25 ppm
NT = Not tested

Table 2. Activity of Compounds **1** and **2** at pH 7.0 *vs* Bacteria

Organism	Minimum Inhibitory Concentration, MIC (ppm)		
	1	**2**	Standard[a]
P. fluorescens	<0.13, 0.2*	4	<0.2*
P. aerugenosa	63	>250	NT
E. coli	250	>250	NT
S. aureus	<0.13, 0.2*	>250	NT

[a]4,5-Dichloro-2-*n*-octylisothiazolin-3(2H)-one, a commercial biocide
*Separate test: endpoint 0.2 ppm
NT = Not tested

Table 3. Activity of Anthraquinones **1** and **2** towards *P. ultimum* and *P. capsici*

Compound	*P. ultimum*	*P. capsici*
	EC_{50} (ppm)	MIC (ppm)
1	0.030	0.2
2	3.6	NT
Metalaxyl	0.017	0.4

Table 4. Activity of Anthraquinone **1** vs an Alga and a Cyanobacterium

Organism	Minimum Inhibitory Concentration MIC (ppm)	
	1	Standard[a]
Chlorella pyrenoidosa	<2	0.3
Anabaena flos-aquae	<2	0.1

[a]Kathon 886®: 3:1 w/w ratio of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one

Table 5. Activity of Anthraquinones **1**, **3**, and **4** *vs P. fluorescens* as a Function of pH

pH	Minimum Inhibitory Concentration, MIC (ppm)			
	1	**3**	**4**	Standard[a]
4.0	0.2*	NT	NT	50*
5.5	0.025	>100	100	NT
7.0	0.05, 0.2*	25	50*	0.2
8.0	0.05	50	0.4	NT
9.0	0.05,0.2*	50	0.2	50*

[a]Iodopropynyl N-*n*-butyl carbamate, a commercial biocide
*Identical experiment, with trypticase soy broth medium
NT = Not tested

Method for Antifungal Assay (Table 1)

A suspension of about 10^4 organisms/ml were made for *Aspergillus niger*, *Aureobasidium pullulans*, *Cladosporium resinae*, *Chaetomium globosum* and *Rhodotorula rubra*; 2 ml of each suspension was diluted into 100 ml of Mycophile® broth growth medium at pH 5.0. For each organism and compound, 190 µl of the cell suspension was placed in the first well of each row in a flat-bottomed 96-well microtiter plate; 100 µl of the cell suspension was added to each of the other wells, except that the last one in each row was left vacant. Test compounds were dissolved in DMSO at 4 mg/ml, and diluted 20-fold into Mycophile® broth growth medium at pH 5.0. Two-fold serial dilutions were made for each compound and organism. Plates were incubated at 30 °C for 7 days, and MICs were determined by visual inspection of fungal growth. The standard was iodopropynyl N-*n*-butyl carbamate (IPBC), a commercial biocide.

Method for Antibacterial Assay (Table 2)

Suspensions of about 10^4 organisms/ml were made for *Escherichia coli, Pseudomonas aerugenosa, Pseudomonas fluorescens, and Staphylococcus aureus*; 2 ml of each suspension was diluted into 100 ml of liquid trypticase soy broth (Difco) growth medium at pH 7.0. Test compounds were dissolved in DMSO at 4 mg/ml, and diluted 20-fold into liquid trypticase soy broth (Difco) growth medium at pH 7.0. Two-fold serial dilutions were made for each compound and organism. Plates were prepared as given above for the antifungal assay, then were incubated at 37 °C for 2 days. MICs were determined by visual inspection of fungal growth. 4,5-Dichloro-2-*n*-octylisothiazolin-3(2H)-one, a commercial biocide, was included as a standard.

Method for Antifungal assay *vs. Pythium ultimum* (Table 3)

A dilution series of each compound was prepared in dimethylsulfoxide (DMSO), and 0.1 ml of each dilution was added to 19.9 ml of liquid asparagine-sucrose medium[5] in 9 cm diameter petri dishes to give the desired final concentrations of test compound. Plates were inoculated with mycelial plugs, 7 mm diameter, taken from the growing edge of 48 h old cultures of *P. ultimum*, (ATCC 26083), grown on potato dextrose agar. The increase in mycelial dry weight was determined after growth for 48 h at 25 °C with shaking at 60 rpm. EC_{50} values were calculated from dose response curves. Metalaxyl was included as a standard.

Method for Antifungal assay *vs. Phytophthora capsici* (Table 3)

Test compounds were dissolved in DMSO at 4 mg/ml, and diluted 20-fold into liquid asparagine-sucrose medium. Two-fold serial dilutions were prepared in flat-bottomed 96-well microtiter plates containing 100 µl of medium per well. A suspension of *P. capsici* zoospores was prepared[6] at 5 X 10^4 zoospores/ml and 100 µl added to each well. Plates were incubated at 25 °C for 48 h, and MICs were determined by visual inspection of fungal growth. Metalaxyl was included as a standard.

Method for Antialgal and Cyanobacterium Assay (Table 4)

A suspension of about 10^4 organisms/ml were made for the green algae *Chlorella pyrenoidosa* and the cyanobacterium *Anabaena flos-aquae*; 2 ml of each suspension was diluted in 100 ml of synthetic cooling tower water at pH 8.5. Test compounds were dissolved in DMSO at 4 mg/ml, and diluted 20-fold into liquid synthetic cooling water medium at pH 8.5. Two-fold serial dilutions were made for each organism. Plates were prepared as described for the antifungal assay above and were incubated on a shaker under fluorescent light at 25 °C for 7 days. MICs were determined by visual inspection of turbidity. A 3:1 w/w ratio of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one (Kathon 886®), a commercial biocide, was included as a standard.

The composition of synthetic cooling water medium is as follows. Mineral salts, ppm (concentration in water): $CaCl_2$-H_2O, 642; $MgCl_2$-$2H_2O$, 487; Na_2CO_3, 233; K_2SO_4, 290; $CuCl_2$-$2H_2O$, 0.7; EDTA disodium salt, 2.7; $FeCl_3$, 1.9; Nutrients, ppm: Glucose, 50; NH_4NO_3, 57; K_2HPO_4, 23; Scale and Corrosion Inhibitors, ppm: Acrylate Polymer [Acumer® 2000 (Rohm and Haas Company], 5; Phosphonate [Bayhibit® AM (Mobay Chemical Company)], 5; Tolyltriazole [Cobratec® TT-50-S (Sherman and Williams Chemical Company)], 2.

Method for Antibacterial Assay *vs. P. fluorescens* as a Function of pH (Table 5)

Aliquots of Mycophil® broth were adjusted to pH 5.5, 7.0, 8.0 or 9.0 with either 1N HCl or 1N KOH, and then autoclaved for 15 min at 115 °C. After cooling, 100 ml aliquots of the media were inoculated with 75 ml of a cell suspension of about 10^6 *Pseudomonas fluorescens*/ml. Test compounds were dissolved in DMSO at 4 mg/ml, and diluted 20-fold into Mycophil® broth adjusted to pH 5.5, 7.0, 8.0 or 9.0. Two-fold serial dilutions were made for each compound and pH. The plates were prepared as described for the antifungal assay and were incubated at 37 °C for 24 h. MICs were determined by visual inspection of turbidity. Iodopropynyl N-*n*-butyl carbamate (IPBC), a commercial biocide, was included as a standard. Control wells with no compound added showed microbial growth by visual inspection.

Method for Tobaccco root assay

Inhibition of tobacco root growth was assayed according to the methods given in reference 6. Trifluralin and chlorpropham were included as standards.

Acknowledgement. This investigation was supported in part by a grant from the National Cancer Institute (CA 17625) awarded to K.H. Lee.

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(*Received in USA* 11 *August* 1997; *accepted* 10 *November* 1997)