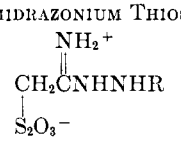
TABLE I
α-ACETAMIDRAZONIUM THIOSULFATES



Compd	R	% yield	Mp, °C dec	Formula[a]	Antiradiation act. Approx LD_{50}, mg/kg	Drug dose, mg/kg[d]	30-day survival, %[e]
III	C_6H_5	13	152–153	$C_8H_{11}N_3O_3S_2$			
IV	p-$O_2NC_6H_4$	37	178–179	$C_8H_{10}N_4O_5S_2$[b]	150	80	20
V	o-$CH_3OC_6H_4$	19	136–137	$C_9H_{13}N_3O_4S_2$	110	25	13
VI	p-FC_6H_4	19	143–144	$C_8H_{10}FN_3O_3S_2$			
VII	EtO_2CCH_2	17	143–144	$C_6H_{13}N_3O_5S_2$[c]	750	400	13

[a] Analytical results obtained for C, H, and N were within ±0.4% of the theoretical values unless listed otherwise. [b] N: calcd, 18.3; found, 17.8. [c] C: calcd, 26.6; found, 26.1. [d] Compounds were suspended in a physiological saline solution containing 0.3% carboxymethylcellulose and 0.1% Tween 80 and administered intraperitoneally to mice, which were subjected to lethal radiation of 950 R. [e] No survival among control mice.

was triturated with boiling absolute EtOH and dried to give 2.9 g (37%) of orange product, mp 178–179° dec.

Acknowledgments.—The authors are indebted to Drs. D. P. Jacobus and T. R. Sweeney for antiradiation data and to Mr. P. E. Olson, Mr. G. J. Lillquist, and the members of the Analytical Research and Services Laboratory of the 3M Company for microanalytical and spectral analyses.

5-Nitro-8-quinolinols and Their Copper(II) Complexes. Implications of the Fungal Spore Wall as a Possible Barrier against Potential Antifungal Agents[1]

HERMAN GERSHON, MAYNARD W. MCNEIL, AND YVONNE HINDS

Boyce Thompson Institute for Plant Research, Yonkers, New York 10701

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A hypothesis was proposed by Gershon, *et al.*,[2,3] which suggested that the fungal spore wall acted as a barrier against certain potential antifungal agents. If the geometry and charge distribution of a molecule are not compatible with geometry and distribution of charge around the periphery of the holes in the fungal spore wall, the compound cannot penetrate the wall and cause toxic reactions in the spore. It was deduced from the shapes and dimensions of the Cu(II) chelates of substituted 8-quinolinols that the holes cannot be circular but may be elliptical or hexagonal.

If the hypothesis is sound, and the explanation of the nontoxicity of certain compounds is due to the long axes being greater than the major axes of the spore holes, alteration of a secondary axis of the compound should not cause the derivative to become toxic. Bis(5-nitro-8-quinolinolato)copper(II) was shown to be nontoxic to five fungi, *Aspergillus niger*, *Trichoderma viride*, *Aspergillus oryzae*, *Myrothecium verrucaria*, and *Trichophyton mentagrophytes*.[2] The explanation was that its long axis was greater than the diameter of the holes in the spore walls and penetration of the spores could not be effected. Consequently, any 7-substituted 5-nitro-8-quinolinol Cu(II) complexes should also be nontoxic to the same fungi. To test this, the Cu(II) bischelates of 7-fluoro-, 7-chloro-, 7-bromo-, and 7-iodo-5-nitro-8-quinolinol were prepared and screened against the same five fungi.

Although the chloro ligand[4] was previously prepared by a Skraup synthesis, the present preparation was obtained by treatment of 5-nitro-8-quinolinol[5] with NaOCl. The bromo[6] and iodo[7] compounds were also prepared from 5-nitro-8-quinolinol. The respective copper(II) complexes were prepared from the ligands by treatment with cupric acetate in aqueous MeOH, or aqueous MeOH containing DMF.

The data characterizing the new compounds are contained in Table I. All of the compounds were screened for antifungal activity in shake culture against the spores of the five fungi previously mentioned, according to published methods.[8]

The data of Table II show that the 7-substituted 5-nitro-8-quinolinols possess significant antifungal activity but weaker than that of the parent compound, 5-nitro-8-quinolinol.[2] The Cu(II) bischelates were all inactive. Thus, these results were found to be consistent with our hypothesis.[2,3]

It should be mentioned that in certain cases the freshly prepared chelate caused inhibition of mycelial development. On repeated boil-up of the chelate with DMF, the inhibitory effect was eliminated. Upon cooling the DMF solution, a chelate was obtained which was inhibitory. When both the soluble and insoluble chelates were decomposed with H_2S and the Cu(II) removed, followed by recovery of the ligands, gas chromatography of the trimethylsilyl derivatives indicated that both chelates appeared to possess the same component parts. We cannot interpret this observation properly, but a reasonable explanation may be that in the formation of the chelate, a small amount of *cis*

(1) This work was supported in part by the U. S. Public Health Service, Grant No. AI-05808.

(2) H. Gershon, R. Parmegiani, A. Weiner, and R. D'Ascoli, *Contrib. Boyce Thompson Inst.*, **23**, 219 (1966).

(3) H. Gershon, *J. Med. Chem.*, **11**, 1094 (1968).

(4) F. X. Wiederkehr and E. Hofstetter, *Helv. Chim. Acta*, **35**, 468 (1952).

(5) V. Petrow and B. Sturgeon, *J. Chem. Soc.*, 570 (1954).

(6) H. Vogt and P. Jeske, *Arch. Pharm.*, **291**, 168 (1958).

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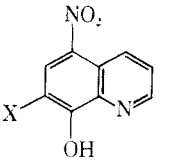
(8) H. Gershon and R. Parmegiani, *Appl. Microbiol.*, **11**, 62 (1963).

TABLE I

7-HALOGENO-5-NITRO-8-QUINOLINOLS AND COPPER(II) COMPLEXES

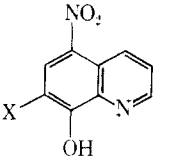


Compound	X	Yield, %	Mp, °C dec[a]	Formula	Analyses
			8-Quinolinols		
Ia[b]	F	90	220–221	$C_9H_5FN_2O_3$	C, H, F, N
Ib	Cl	93	240–242[f]		
Ic	Br	95	192[g]		
Id[d]	I	85	257–258[h]		
			Bis(8-quinolinolato)copper(II) Complexes		
IIa[c]	F	98	>400	$C_{18}H_8F_2N_4O_6Cu$	C, H, N
IIb[c]	Cl	88	>400	$C_{18}H_8Cl_2N_4O_6Cu$	C, H, N
IIc[c]	Br	70	>400	$C_{18}H_8Br_2N_4O_6Cu$	C, H, N
IId[e]	I	98	>400	$C_{18}H_8I_2N_4O_6Cu$	C, H, N

[a] Analytical sample. [b] From MeOH. [c] From MeOH–DMF. [d] From DMF. [e] Boiled in DMF, insoluble portion used as analytical sample. [f] Lit.[4] mp 235°. [g] Lit.[5] mp 180°. [h] Lit.[6] mp 249°.

TABLE II

MINIMAL ANTIFUNGAL ACTIVITY OF 5-NITRO-7-HALOGENO-8-QUINOLINOLS AND DERIVED Cu(II) COMPLEXES



		Min antifungal act., μmoles/l.									
		A. niger		*T. viride*		*A. oryzae*		*M. racemosus*		*T. mentagrophytes*	
Compound	X	S[a]	C[a]	S	S	S	C	S	C	S	C
					8-Quinolinols						
Ia	F	0.29	NA[b]	0.021	0.13	0.39	NA	0.034	0.087	<0.0048	0.0096
Ib	Cl	0.43	NA	0.067	0.090	0.22	0.45	0.043	0.022	<0.0045	<0.0045
Ic	Br	0.097	0.34	0.048	0.052	0.22	NA	0.019	0.019	<0.0037	<0.0037
Id	I	0.19	NA	0.088	0.095	NA		0.019	0.044	<0.0032	<0.0032
					Bis(8-quinolinolato)copper(II) Complexes						
IIa	F	NA		NA		NA		NA		NA	
IIb	Cl	NA		NA		NA		NA		NA	
IIc	Br	NA		NA		NA		NA		NA	
IId	I	NA		NA		NA		NA		NA	

[a] S = fungistatic, C = fungicidal. [b] NA = not active below 100 ppm.

complex is produced along with the major portion of *trans* compound. The *cis* complex would have a shorter long axis than the *trans* form and also a dipole which would not force it to align parallel with the hole of the spore wall on approaching it[2] prior to penetration.[9]

Experimental Section[10]

7-Fluoro-8-quinolinol. 7-Amino-8-quinolinol[3] as the hemi-

(9) It has been suggested by a referee that the work of C. F. Marks, I. J. Thomason, and C. E. Castro, *Exp. Parasitol.*, **22**, 321 (1968), be considered with respect to our hypothesis on the fungal spore wall as a possible barrier against potential antifungal agents. These authors attempted to implicate a subcuticular membrane as the actual barrier to penetration in nematodes rather than the cuticle itself. Although this study deals with the rates of penetration of the animal organism by nematocides and other compounds, no work was reported on the nontoxicity of potential nematocides. The reasons for our choice of fungal spore wall as the barrier against the class of compounds in our study rather than the membrane are presented in ref 2 and 3. It should be noted that we hold that membrane carries out the usual functions attributed to it, but that wall also acts as a barrier of a sort.

(10) Melting points were taken in a Mel-Temp melting point apparatus and are uncorrected.

sulfate (4.2 g, 0.02 mole) was suspended in 40 ml of THF with magnetic stirring. Fluoroboric acid (48–50%, 18 ml) was added, and the temperature was maintained at 0–5°. Solid $NaNO_2$ (1.42 g, 0.022 mole) was added in small portions over 0.5 hr after which stirring was continued for 2 hr longer. The diazonium fluoroborate was removed by filtration on a coarse sintered-glass funnel, slurried twice with 5-ml portions of cold 1:1 Et_2O–EtOH (v/v) and twice with 10-ml portions of cold Et_2O, and dried under vacuum at 30–35° overnight. The yield of product was 4.25 g (86% calculated as the monofluoroborate), mp 177–179° dec. The compound (4.2 g, 0.016 mole) was spread in a thin layer over the bottom of a Fernbach flask fixed with an air condenser. The layer of dry material was combusted with a flame, boiled with 40 ml of 5% H_2SO_4, filtered, brought to pH 5 with dilute NaOH, and steam distilled. Upon filtration of the distillate, a yield of 0.5 g of product was obtained, mp 108–109°. Ether extraction of the filtrate yielded an additional 0.075 g of material, mp 100°. The combined yield of 7-fluoro-8-quinolinol was 22%. An analytical sample was obtained by crystallization from EtOH, mp 110–110.5°. *Anal.* (C_9H_6FNO) C, H, F, N.

7-Fluoro-5-nitro-8-quinolinol (Ia).—A solution of 10.0 g (0.061 mole) of 7-fluoro-8-quinolinol in a mixture of 150 ml of AcOH and 40 ml of DMF was cooled to 5°. A mixture of 5.0 ml (0.79 mole) of 70% HNO_3 in 20 ml of AcOH was added dropwise with agitation. The cooling bath was removed, and stirring was

continued. When the temperature rose to 15–20°, all of the insoluble material went into solution. The clear solution was poured into 500 ml of H_2O and the mixture was adjusted to pH 5. The product was removed by filtration, washed with deionized H_2O, and dried overnight at 70°. The crude product melted at 219–220° dec.

Bis(7-fluoro-5-nitro-8-quinolinolato)copper(II) (IIa).—Two solutions containing 0.41 g (0.0018 mole) of 7-fluoro-5-nitro-8-quinolinol in 10 ml of MeOH and 0.21 g (0.0010 mole) of $Cu(OAc)_2 \cdot H_2O$ in 20 ml of 75% aqueous MeOH were mixed and stirred for 1 hr. The product was removed by filtration, boiled in DMF several times, and the insoluble material was washed with acetone and dried under vacuum at 100° overnight. The compound was sufficiently pure for analysis.

7-Chloro-5-nitro-8-quinolinol (Ib).—To 400 ml of H_2O was added 5.7 g (0.028 mole) of 5-nitro-8-quinolinol[4] and 1.8 g (0.026 mole) of 85% KOH. Solution was effected by stirring and heating. After cooling to 30°, 50 ml of NaOCl (5.25%) was added and stirring was continued 1 hr longer. The mixture was brought to pH 5 with 25 ml of AcOH. The product was obtained by filtration, washing with deionized H_2O, and drying at 70° overnight.

6,6′-Dithiobispurinyl Nucleosides[1]

FRED KELLER AND JAMES E. BUNKER

Medicinal Chemistry Section, Chemical Research Department, Riker Laboratories, Division of Dart Industries, Inc., Northridge, California 91324

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A number of nucleosides of purine-6-thiol (**1**) and 2-aminopurine-6-thiol (**2**) have been prepared in an effort to modify the toxicity of these clinically useful anticancer agents and to provide drugs for use against **1**- and **2**-resistant cancer strains.[2] A serious problem which has presented itself in the use of these new drug candidates is their rapid elimination and metabolism by oxidative pathways to therapeutically inactive products. The metabolism of 6-mercaptopurine and some of its derivatives has been the subject of a recent review.[3]

In an attempt to provide a "reservoir" source of **1** and **2** as well as their β-D-ribofuranosides, Doerr, *et al.*,[4] prepared the disulfides of these compounds using an adaptation of the iodine-buffer procedure of Miller, *et al.*[5] The disulfides could be envisioned as reverting to the corresponding thiol and sulfenic acid through chemical means by the attack of base,[4] or alternately an enzymatic system such as that regulating the oxidation and reduction of glutathione could prevail to reduce the disulfide to the thiol.

The earlier paper[4] did not record any metabolic data to support directly the "reservoir" hypothesis but did note that the activity of the disulfide of thioinosine in the Sarcoma 180 system was greater than that of **1** while the corresponding thiol was inactive. The thioguanosine disulfide was also found to be a more potent inhibitor on a molar basis than either **2** or thioguanosine.

Despite these rather intriguing results there does not appear to have been any further study upon the effects of disulfide formation on biological activity in 6-thiopurinyl nucleosides.

In an effort to explore this aspect further we have prepared the disulfides of several 6-thiopurinyl nucleosides. The candidates chosen for conversion to their disulfides were α- and β-9-(2-deoxy-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[2] (**3**), α- and β-2-amino-9-(2-deoxy-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[6] (**4**), 9-β-D-arabinofuranosyl-9H-purine-6-thiol[7] (**5**), and 2-amino-9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-9H-purine-6-thiol[8] (**6**). All of the compounds chosen with the exception of α-**3** have previously demonstrated significant activity in the leukemia L1210 test system[9] (Table I).

TABLE I
ACTIVITY OF STARTING NUCLEOSIDES AND DISULFIDES IN THE L1210 SCREEN

	—SH[a,8]—		—S-S[b]—	
Compound	Dose, mg/kg	ILS, %	Dose, mg/kg	ILS, %
α-3			400	16
β-3	200	56	4–600	30
α-4	15	95	400	47
β-4	20	99	150	32
β-5	400	63	400	40
β-6	200	89	75–150	33

[a] Optimal dose. [b] Optimal dose not determined; administered intraperitoneally in saline suspension.

The thiols were smoothly converted to disulfides in good yields upon treatment in the iodine-buffer system.[4,5]

With the exception of α- and β-**3** all of the nucleoside disulfides proved to be quite water insoluble and were recovered by filtration of the reaction mixture and conveniently purified by recrystallization from DMF–H_2O (method A). The 2-deoxynucleoside disulfides (α- and β-**3**) had significant water solubility and were best recovered by lyophilization of the oxidation mixture and recrystallization of the residues from a minimum of H_2O (method B). The physical constants of the new compounds are listed in Table II. All of the disulfides exhibited the characteristic uv spectra described previously.[4,10]

Biological Testing.—The preliminary screening results in the L1210 system for the new disulfides are listed in Table I along with the optimal doses for the parent thiols.[9] It would appear from these data that all of the

(1) Most of this work was performed under the auspices of the Cancer Chemotherapy National Service Center, National Institutes of Health, Public Health Service, under Contract PH-43-66-904. The views expressed in this paper are those of the authors and do not necessarily reflect those of the CCNSC.

(2) See R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **27**, 3949 (1962), and leading references therein for a discussion of the rationale for the preparation of these compounds.

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(9) A. Goldin, H. B. Wood, Jr., and R. R. Engle, *Cancer Chemotherapy Rept., Part 2*, **1**, 1 (1968).

(10) The uv spectra of the nucleoside disulfides showed the decomposition in base described previously.[4] Two peaks were evidenced in the base spectrum of **5**-S-S, one at ~312 mμ previously[4] attributed to the chromophore **5** and the second at ~355 mμ assigned[4] to an unknown component. Recent work on the base treatment of bis(4-thiouridine) disulfide [B. C. Pal, M. Uziel, D. G. Doherty, and W. E. Cohn, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, Paper 213] makes it appear likely that the long-wavelength absorption is due to the presence of the nucleoside sulfenic acid.