Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. The ^1H NMR spectra were recorded on a Varian EM390 spectrometer with Me_4Si as internal reference. Solvent was removed under vacuum (rotovap). Thin-layer chromatography was performed on precoated Analtech silica gel sheets GHLF which were developed with 15% MeOH in CH_2Cl_2 and visualized by UV, I_2, or Fe(III)/MeOH spray. Microanalyses were performed by the Analytical Services, Chemistry Department, University of California, Berkeley. Diacetoxybenzoyl chloride was prepared by the method of Bergeron.[12] Methyl 2,3-Dimethoxy-4-(chloroformyl)benzoate was prepared by the method of Weitl.[3]

Kinetics. Apotransferrin (Sigma Chemical Co.) was saturated by the procedure of Bates et al. with a fresh $Fe(NTA)_2^{3-}$ solution followed by gel filtration on two preequilibrated Sephadex G-25 resins eluted with 0.1 M $NaClO_4$ and 0.1 M Tris-HCl (pH 7.4).[13] The ratios $A_{280}/A_{466} \leq 24$ and $A_{428}/A_{466} = 0.85$ indicate ≥95% saturation. Ligand solutions in 0.1 M Tris-HCl (pH 7.4) were freshly prepared by heating slightly until the ligand dissolved and then cooled to 25.0 °C. Half-milliliter aliquotes each of ligand and diferric transferrin solutions were mixed in a thermostated quartz cell maintained at 25.0 °C. Visible spectra were taken on a Hewlett Packard 8450A UV/Vis spectrophotometer. Linear plots of $-\ln(A_t - A_\infty)/(A_0 - A_\infty)$ vs. time at 426 nm were obtained over 2 half lives. The value of k_{obsd} was obtained by linear least-squares analysis.

***N*-(2,3-Dihydroxybenzoyl)desferrioxamine B (2).** Solutions of 2,3-diacetoxybenzoyl chloride (8.784 g, 34 mmol) in 400 mL of CH_2Cl_2 and 500 mL of saturated $NaHCO_3$ were added simultaneously over 0.5 h to a vigorously stirred solution of Desferal (5.00 g, 8 mmol) in 75 mL of water on an ice bath. After 1.5 h an additional 100 mL of saturated $NaHCO_3$ was added. Upon completion of the reaction after 2.5 h, 800 mL of CH_2Cl_2 was added, and the layers were separated. The aqueous layer was extracted with two 100-mL portions of CH_2Cl_2, and then the combined organic layers were washed with two portions of 0.5 M HCl (250 mL) and dried (Na_2SO_4). Evaporation of the solvent yielded a foam, which was then dissolved in 250 mL of MeOH, degassed in vacuo, and put under N_2. The solution was saturated with NH_3 gas for 10 min and then evaporated. To remove NH_3, methanol was added to the residue and then removed under vacuum (several times). Recrystallization from MeOH afforded 4.389 g (85%) of off-white powder: mp 178–178.5 °C; ^1H NMR (Me_2SO-d_6) δ 1.0–1.8 (br m, 18 H), 1.9 (s, 3 H), 2.3 (br t, 4 H), 2.6 (br t, 4 H), 2.9–3.2 (br m, 4 H), 3.2–3.7 (br m, 8 H), 6.7 (t, 1 H), 6.95 (d, 1 H), 7.3 (d, 1 H), 7.8 (br t, 2 H), 8.8 (br t, 1 H), 9.1 (br s, 1 H), 9.6 (br s, 3 H). Anal. Calcd for $C_{32}H_{52}N_6O_{11}\cdot H_2O$: C, 53.74; H, 7.63; N, 11.76. Found: C, 53.65; H, 7.23; N, 11.64.

***N*-(2,3-Dimethoxy-4-carboxybenzoyl)desferrioxamine B (3).** Solutions of methyl 2,3-dimethoxy-4-(chloroformyl)benzoate (2.189 g, 8 mmol) in 150 mL of ether and 20 mL of 0.5 M NaOH were added simultaneously over 0.5 h to a vigorously stirred solution of Desferal (5.00 g, 8 mmol) and $FeCl_3$ (1.297 g, 8 mmol) in 100 mL of H_2O and 60 mL of 0.5 M NaOH on an ice bath. Over the next 1.5 h, 10 mL of 0.5 M NaOH were added to keep the pH above 9. The layers were separated, and the ether layer was washed with 50 mL of H_2O twice. The combined aqueous layers were reduced in volume by about one-half and then basified with 4 M NaOH to pH 12. The resulting ferric hydroxide was removed by filtration through a Metricel 0.45-μm filter. Acidification was 6 M HCl to pH 2 yielded a white precipitate, which was recrystallized from MeOH to give 5.297 g (94%) of white powder: mp 146.5–148.5 °C; ^1H NMR (Me_2SO-d_6) δ 1.0–1.7 (br m, 18 H), 1.9 (s, 3 H), 2.2 (br t, 4 H), 2.45 (br t, 4 H), 2.7–3.1 (br m, 4 H), 3.1–3.5 (br m, 8 H), 3.75 (s, 6 H), 7.23 (d, 1 H), 7.40 (d, 1 H), 7.7 (br t, 2 H), 8.2 (br t, 1 H). Anal. Calcd for $C_{35}H_{56}N_6O_{13}$: C, 54.64; H, 7.36; N, 10.97. Found: C, 54.82; H, 7.41; N, 10.80.

***N*-(2,3-Dihydroxy-4-carboxybenzoyl)desferrioxamine B (4).** A solution of BBr_3 (0.50 mL, 5.3 mmol) in 20 mL of CH_2Cl_2 was added to **3** (0.9212 g, 1.2 mmol) suspended in 10 mL of CH_2Cl_2 over 5 min while on an ice bath under N_2. After 6 h, 50 mL of H_2O was added, and the reaction mixture was stirred for 2 h. The liquids were decanted. To remove the volatile borates, methanol was added to the gum adhering to the walls of the reaction flask and then removed under vacuum (three times). Recrystallization from MeOH afforded 0.5031 g (57%) of white solid: mp 169–171 °C; ^1H NMR (Me_2SO-d_6) δ 1.0–1.7 (br m, 18 H), 1.9 (s, 3 H), 2.2 (br t, 4 H), 2.5 (br t, 4 H), 2.7–3.1 (br m, 4 H), 3.1–3.5 (br m, 8 H), 7.2 (d, 1 H), 7.36 (d, 1 H), 7.7 (br m, 2 H), 8.8 (br m, 1 H), 9.5 (br m, 2 H). Anal. Calcd for $C_{33}H_{52}N_6O_{13}\cdot H_2O$: C, 52.21; H, 7.19; N, 11.07. Found: C, 51.99; H, 6.88; N, 10.95.

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Registry No. 1, 84010-58-2; 2, 84010-59-3; 3, 84010-60-6; 4, 84010-61-7; Desferal, 70-51-9; 2,3-diacetoxybenzoyl chloride, 65055-19-8; methyl 2,3-dimethoxy-4-(chloroformyl)benzoate, 75956-64-8; ferrioxamine B, 14836-73-8; Fe, 7439-89-6.

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Synthesis of (*E*)-1-(5-Chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-Dichlorophenylhydrazone Hydrochloride, a Novel, Orally Active Antifungal Agent

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The preparation, determination of isomeric configuration, and antifungal properties of (*E*)-1-(5-chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-dichlorophenylhydrazone hydrochloride (1) are described. In vitro, compound 1 has been shown to have activity against *Candida albicans* comparable with miconazole. When administered orally to animals with experimentally induced vaginal candidiasis or systemic candidiasis, compound 1 produced results approaching those produced by ketoconazole. In addition, topical administration of compound 1 to rats with vaginal candidiasis produced results comparable with those produced by similar administration of clotrimazole. Unlike ketoconazole, which is active by a mechanism that is essentially fungistatic, compound 1 shares with miconazole a mode of action that is fungicidal. However, unlike miconazole, compound 1 exhibits activity following oral administration. Compound 1 has been found to be negative in the Ames test.

Ketoconazole[1] is at present the only imidazole-based antifungal agent that can be used orally in the treatment of systemic fungal infections in man. Other imidazole-based antifungal agents, such as miconazole, econazole,[2]

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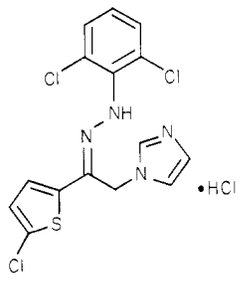
and clotrimazole,[3] have found considerable therapeutic utility as topical agents in cases of vaginal candidiasis and against dermatophyte infections. Miconazole has also been successfully administered intravenously in the treatment of some systemic fungal infections.[4]

There has recently been considerable interest in the mode of action of these drugs; in particular, it has been reported[5] that clotrimazole and miconazole, as well as presumably econazole, which is extremely similar to miconazole structurally, exhibit two modes of action. Ketoconazole only exhibits one of these actions. The mode of action common to each of these drugs is such that at low concentrations they block the demethylation of lanosterol, thereby inhibiting the formation of ergosterol.[6,7] This mechanism is essentially fungistatic. However, Sud and Feingold[5] have reported that at higher concentrations both clotrimazole and miconazole exhibit a rapid fungicidal action that they describe as direct membrane damage (DMD). Ketoconazole has little or no such action and, therefore, has little or no fungicidal activity. These authors suggest that the absence of fungicidal activity may lead to problems with long-term treatment of fungal infections in compromised hosts and also increase the likelihood of the development of resistant strains. To date, resistance has not been a problem with this class of antifungal agents.

Van Cutsem et al.[8] have shown ketoconazole to be fungicidal for 6 of 45 strains of *C. albicans*. However, their initial inoculum was lower (9×10^2) and incubation time longer (7 days) than in the study described below or those of Sud and Feingold.[5] It may be that ketoconazole is fungicidal when in contact with fungi for a prolonged period, but rapid fungicidal activity is likely to be of importance clinically, especially in patients with compromised immune systems.

Thus, there would appear to be a need for an orally active, imidazole-based antifungal agent exhibiting the rapid fungicidal action of miconazole and clotrimazole. The biological properties of (*E*)-1-(5-chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-dichlorophenylhydrazone hydrochloride (**1**), which are given below, suggest that this



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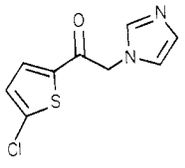
compound meets these requirements. In addition to its in vivo activity, compound **1** has been shown to have rapid fungicidal activity in vitro. Even at levels no greater than the MIC, marked reductions in viability were seen within a few hours of treatment. The results correspond closely with those reported by Sud and Feingold[5] and will be published in detail elsewhere.

Table I. In Vitro Antifungal Activity of Compounds 1 and 3 in Comparison with Miconazole

organism[a]	MIC, mg/L		
	1	3	miconazole
T.m.	12.5	12.5	1.6
T.r.	25	12.5	1.6
E.f.	25	25	12.5
M.c.	25	25	6.2
C.a. (A)	6.2	6.2	6.2
C.a. (B)	6.2	6.2	6.2
C.a. (GB)	12.5	12.5	6.2
C.a. (3153)	12.5	12.5	12.5

[a] *T.m.* = *Trichophyton mentagrophytes*; *T.r.* = *Trichophyton rubrum*; *E.f.* = *Epidermophyton floccosum*; *M.c.* = *Microsporum canis*; *C.a.* (A) = *Candida albicans* A; *C.a.* (B) = *Candida albicans* B; *C.a.* (GB) = *Candida albicans* GB; *C.a.* (3153) = *Candida albicans* 3153.

Chemistry. The hydrazone **1** was prepared by treatment of the known ketone **2**[2] with 2,6-dichlorophenyl-



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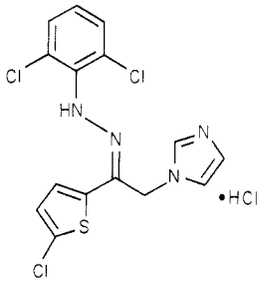
hydrazine hydrochloride under reflux in ethanol for 18 h. Addition of toluene, followed by concentration of the solution and subsequent cooling, afforded the hydrazone **1** as a crystalline hydrochloride salt in good yield. The assignment of stereochemistry was made with the aid of X-ray crystallographic data.[9]

Interestingly, when this hydrazone was dissolved in a mixture of methanol and 1,2-dichloroethane, heated under reflux for 5 h, and then cooled, a product was obtained, albeit in low yield, which was identified as the *Z* isomer (**3**). This isomer reverted to the *E* form on standing at room temperature for several weeks.



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Biological Data. In vitro data obtained by agar dilution assay for compounds **1** and **3** in comparison with miconazole, used as an internal standard, are displayed in Table I. These results, in which the compounds exhibited complete inhibition of fungal growth at the concentration (micrograms per milliliter) indicated, are broadly comparable to those of miconazole against *Candida albicans*,

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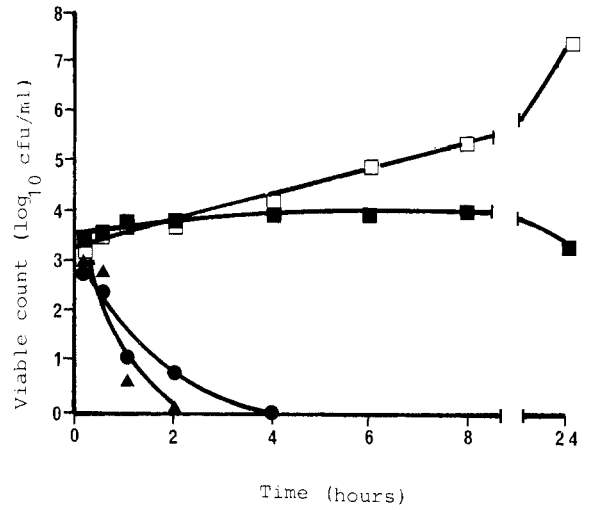

Figure 1. Effect of compound 1, miconazole, and ketoconazole on the viability of *Candida albicans* in vitro: (□) control; (▲) compound 1; (●) miconazole; (■) ketoconazole. Compounds were added to cultures immediately after inoculation at a concentration of 10^{-3} M. Growth was measured by plating dilutions of cultures.

Table II. Effect of Oral and Topical Administration of Compound 1 Ketoconazole or Clotrimazole on the Course of Experimental Vaginitis in the Rat Caused by *C. albicans*

compd	treatment	% reduction in recovery of *C. albicans* compared with control
	Oral Administration	
1	10 mg/kg	47
1	20 mg/kg	63
ketoconazole	10 mg/kg	68
	Topical Administration	
1	1% w/v	54
clotrimazole	1% w/v	75

although inferior against dermatophytes.

The effect of compound 1 on the viability of *C. albicans* in comparison with miconazole and ketoconazole was determined, and the results are summarized in Figure 1. Both compound 1 and miconazole were rapidly fungicidal in vitro at 10^{-3} M. At this concentration, complete killing had occurred by 2 (compound 1) and 4 h (miconazole). Ketoconazole was clearly fungistatic up to 8 h at this concentration. After 24 h, there was some slight indication of fungicidal activity, with the recovered cell populations ($\log_{10}$ cfu/mL) being 3.35 compared to an initial population of 3.55 and a peak of 4.04. However, this effect was very small compared with that observed for compound 1 and miconazole. Fungicidal effects were also shown at 10^{-4} M for these compounds with ketoconazole being again only fungistatic. At 2×10^{-5} M, compound 1 and miconazole were fungistatic, and ketoconazole was ineffective (data not shown).

We determined experimentally the in vivo activity of compound 1 against vaginal candidiasis by inducing vaginal infection, with *Candida albicans*, in rats.[10] The efficacy of compound 1 against systemic candidal infections was assessed by monitoring the clearance of *C. albicans* from the kidneys of infected mice.[11] The results obtained from these investigations are given in Tables II and Table III, respectively.

Table III. Effect of Oral Administration of Compound 1 and Ketoconazole on Recovery of *C. albicans* from Kidneys of Mice with an Experimental Candidal Infection

compd	treatment, mg/kg	% reduction compared with control
1	2.5	9
1	5	43
1	10	65
1	20	44
ketoconazole	10	57

Table IV. Effect of Oral and Topical Administration of Compound 1, Griseofulvin, or Clotrimazole on the Course of Experimental Ringworm Infections in the Guinea Pig Caused by *T. mentagrophytes*

compd	treatment	% reduction in recovery of *T. mentagrophytes* compared with control
	Oral Administration	
1	30	70
griseofulvin	60	90
	Topical Administration	
1	2% w/v	77
clotrimazole	1% w/v	88

The in vivo activity of compound 1 against superficial ringworm infections was assessed with the guinea pig animal model,[12] and the results of this study are presented in Table IV.

Toxicological studies have shown the LD_{50} of compound 1 in male rats to be 1420 mg/kg, and in female rats this figure has been found to be 1250 mg/kg. In addition to this, compound 1 has been found negative in the Ames test.[13] Given the above biological data, the compound 1 is proceeding into development with a view to eventual clinical trial.

Experimental Section

Melting points were determined with a Reichert Thermovar melting point apparatus and are uncorrected. New compounds were routinely analyzed by IR (Perkin-Elmer 197) and NMR (Varian EM-360 or Varian FT80A).

(*E*)-1-(5-Chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-Dichlorophenylhydrazone Hydrochloride (1). 1-(5-Chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone (**2**; 2.27 g, 10.0 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (2.30 g, 10.0 mmol) were dissolved in ethanol (80 mL) and heated under reflux for 18 h. Toluene (80 mL) was added, and the solution was concentrated (to 50 mL) and then cooled to afford 1: yield 3.1 g (68%); mp 186–188 °C; NMR (Me_2SO-d_6) δ 5.77 (s, 2 H), 7.7–8.4 (m, 8 H), 9.30 (s, 1 H), 9.75 (s, 1 H). Anal. ($C_{15}H_{12}Cl_4N_4S$) C, H, N. The *E* stereochemistry was confirmed by X-ray crystallographic analysis.

(*Z*)-1-(5-Chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-Dichlorophenylhydrazone Hydrochloride (3). (*E*)-1-(5-Chlorothien-2-yl)-2-(1*H*-imidazol-1-yl)ethanone 2,6-dichlorophenylhydrazone hydrochloride (2 g, 4.8 mmol) was dissolved in methanol (30 mL) and heated under reflux for 10 min. 1,2-Dichloroethane (500 mL) was then added, and the solution was heated under reflux for 5 h. On cooling, the solution was filtered, and the filtrate was evaporated to dryness at 40 °C under reduced pressure. The residue was stirred with 1,2-dichloroethane (50 mL)

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and filtered again, and the filtrate was evaporated to dryness at 40 °C, under reduced pressure to give **3** as an amorphous solid, which was recrystallized from dichloroethane: yield 330 mg (8%); mp 147 °C dec; NMR (Me_2SO-d_6) 5.53 (s, 2 H), 7.0–7.9 (m, 8 H), 8.40 (s, 1 H), 9.29 (s, 1 H). Anal. ($C_{15}H_{12}Cl_4N_4S$) C, H, N.

Biological Methods. Determination of Minimum Inhibitory Concentrations. Minimum inhibitory concentrations (MICs) were determined by agar dilution in Sabouraud dextrose agar in 96-well microtitre plates (Sterilin). Aliquots of drug inocula and medium were added to the wells of the plate with an eight-channel pipet (Titertek). Drugs were dissolved in Me_2SO to give a final concentration of 200 μg/mL. From this stock solution, serial twofold dilutions were made in the wells of the microtitre plate. Final concentrations ranged from 0.8 to 100 μg/mL, and the final concentration of Me_2SO was 1%. For *C. albicans*, the initial inoculum was 10^4 cells/mL. Mycelial inocula were used for dermatophyte species. MICs were read after incubation for 24 h at 37 °C (*C. albicans*) or after 5 days (dermatophytes) at 40 °C. The MIC was taken as the highest dilution of drug at which there was no visible growth. Assays were performed in duplicate.

Determination of the Effect of Compound 1 on the Viability of *Candida albicans*. Aliquots (100 mL) of Sabouraud dextrose broth in conical flasks (250 mL) were inoculated with samples from an overnight culture of *C. albicans* to give an initial population of 10^4 cells/mL. Compound **1**, miconazole, or ketoconazole was added to each flask to give a final concentration of 2×10^{-5} M, 10^{-4} M, and 10^{-3} M. Cultures were incubated at 37 °C on an environmental shaker (L.H. Engineering, Stoke Poges, U.K.), and samples were taken at intervals for determination of viable counts. Diluent was added to each sample prior to culture to ensure adequate dilution of drug carried over. Flasks were prepared in duplicate, and cultures containing no drug were used as negative controls.

Determination of in Vivo Activity against Vaginal Candidiasis in the Rat. Female Wistar rats (6 × 100 g per group) were hysterectomized and ovariectomized. After 3 weeks, and subsequently every 3 days during the experiment, they were injected, subcutaneously, with estradiol diacetate (125 μg) to induce and maintain pseudoestrus. Two days after hormone treatment, estrus was confirmed, and the animals were infected by intravaginal inoculation of 10^7 cells of *C. albicans* on 2 successive days. The infection was allowed to establish for an additional 2 days before treatment commenced. Each animal was considered maximally infected if a colony count of greater than 600 was obtained from 50 μL of vaginal washings at the start of each experiment. Compound 1 was administered orally (by gavage), with ketoconazole at 10 mg/kg used as a positive control. This model was also used to assess the efficacy of compound 1 topically against this infection, with clotrimazole as a positive control. Compound 1 and clotrimazole were each dissolved as 1% solutions [(w/v) in polyethylene glycol 200], and 0.1 mL of these solutions were administered once daily intravaginally for 9 days.

Determination of in Vivo Activity against Systemic Candidal Infection in the Mouse. The mice (10 per group) were infected intravenously with a sublethal dose (50×10^4 cells) of *C. albicans* and treated orally (by gavage) at 0 and 24 h following infection with compound 1 at 20, 10, 5, or 2.5 mg/kg or with ketoconazole at 10 mg/kg. Two days after infection the mice were sacrificed, their kidneys were excised, weighed, and homogenized, and the homogenates were diluted and then inoculated on to glucose–peptone agar. The plates were incubated for 24 h at 37 °C, the resultant colonies were counted, and the numbers were compared with those obtained from infected, untreated controls. The mean yeast count for untreated control mice was 3.5×10^4 per gram per kidney.

Determination of in Vivo Activity against Superficial Ringworm Infections in the Guinea Pig. The shaved backs of female guinea pigs (eight per group) were infected with a suspension of the spores of *Trichophyton mentagrophytes* in saline. The site of inoculation was occluded for 5 days to allow the infection to become established. Each guinea pig before treatment yielded greater than 1000 dermatophyte colonies on culture of skin swabs from the infected area. Treatment was commenced on the 6th day following infection and continued for 8 days. A 1% solution [(w/v) in polyethylene glycol 200] of clotrimazole was used as a positive control for topical application, griseofulvin at a dosage of 60 mg/kg was used as a positive control for oral administration, and the course of disease was followed by cultural studies on skin and hair samples from on and around the sites of infection.

Acknowledgment. The authors thank S. Carrick, S. C. Gyselynck, J. Jones, and Y. S. Lee for assistance in gathering the biological data.

Registry No. (*E*)-**1**, 80168-44-1; **2**, 27088-04-6; (*Z*)-**3**, 80168-45-2; 2,6-dichlorophenylhydrazine hydrochloride, 50709-36-9.

Synthesis and Antitumor Activity of 2-β-D-Ribofuranosylselenazole-4-carboxamide and Related Derivatives

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Treatment of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-carbonitrile with hydrogen selenide provided 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide (**3**). Compound **3** was treated with ethyl bromopyruvate to provide ethyl 2-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)selenazole-4-carboxylates, which after ammonolysis were converted to 2-β-D-ribofuranosylselenazole-4-carboxamide (**6**) and its α-analogue **7**, respectively. Acetylation of nucleoside **6** provided 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)selenazole-4-carboxamide, and phosphorylation of **6** provided the corresponding 5′-phosphate **9**. Compounds **6** and **9** were found to be cytotoxic toward P388 and L1210 cells in culture and effective against Lewis lung carcinoma in mice.

Currently available chemotherapeutic agents have shown no major impact on median survival of patients with bronchogenic and lung carcinoma[1] when used individually or in combination; therefore, more effective antitumor agents are required for chemotherapy. Recently, we have reported the synthesis[2] and mechanism of action[2,3] of 2-

* Address correspondence to Oak Ridge National Laboratory.

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