2-[(Octahydroazocin-2-ylidene)amino]acetophenone Hydrochloride (IIIb). To 50.0 g (0.292 mol) of powdered α-aminoacetophenone hydrochloride (Ia·HCl) was added 50 ml (*ca.* 0.35 mol) of *O*-methylenantholactim (IId); the reactants were thoroughly mixed and were allowed to stand at room temperature for 4 days. (After 3 days, 25 ml of anhydrous EtOH was added.) The mixture was cooled ($-20°$) and the precipitate was collected and washed with anhydrous Et_2O to give 71.0 g of product of mp 149–155°. Two recrystallizations from MeOH–Me_2CO resulted in 41.7 g (51%) of IIIb·HCl (Table I).

To a solution of 10.0 g of IIIb·HCl in MeOH was added 1 equiv of methanolic KOH, the precipitating KCl was filtered off, and the filtrate was evaporated to dryness. The resulting residue was recrystallized three times from MeOH–Me_2CO to give 1.7 g of IVd (Table I). In a duplicate experiment, the residue was reacidified with 1 equiv of methanolic HCl and the resulting salt was recrystallized twice from MeOH–Me_2CO to give 2.6 g of IIIb·HCl (not IVd·HCl).

6,7,8,9-Tetrahydro-2-methyl-3-phenyl-5*H*-imidazo[1,2-*a*]azepine Hydrochloride (Vc). A solution of 22.3 g (0.0795 mol) of IVe in 60 ml of concentrated HCl was refluxed for 3 hr. The mixture was evaporated to dryness under reduced pressure and the residue was recrystallized twice from MeOH–Me_2CO to give 12.8 g (62%) of Vc (Table I): nmr (D_2O) δ 2.30 (s, 3).

α-[(Hexahydroazepin-2-ylidenamine)methyl]benzyl Alcohol Hydrochloride (VIa). A. From 2,3,6,7,8,9-Hexahydro-3-phenyl-5*H*-imidazo[1,2-*a*]azepin-3-ol Hydrochloride (IVc). To 5.0 g of $NaBH_4$ in 100 ml of absolute EtOH at room temperature was added 5.0 g (0.0187 mol) of IVc·HCl (Table I, prepared from Ia·HCl and IIc as described for IVe) and the resulting mixture was stirred for 3 hr. The reaction mixture was then poured into 300 ml of H_2O; the solution was acidified with 2 *N* HCl to destroy excess reagent and made basic again with 2 *N* NaOH. The product was extracted into Et_2O, the extract was washed (4 × H_2O) and dried (Na_2SO_4), ethereal HCl was added, and the solvent was evaporated. The residue crystallized after trituration with Me_2CO, 1.5 g (30%), mp 168–173° dec; the material was identical (mixture melting point and ir) with VIa prepared by method B.

B. From 2-Amino-1-phenylethanol Hydrochloride (VIIa) and IIc. A mixture of 94.4 g (0.543 mol) of VIIa·HCl, 100 ml (0.7 mol) of IIc, and 50 ml of EtOH was allowed to react at room temperature with cooling in an ice bath and was allowed to stand at room temperature for 3 days. The mixture was cooled ($-20°$) and the precipitate was collected, washed with anhydrous Et_2O, and recrystallized twice from MeOH–Me_2CO to give 120.8 g (83%) of VIa (Table I).

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Aryl 5-Nitro-2-furyl Ketone Antifungal Agents

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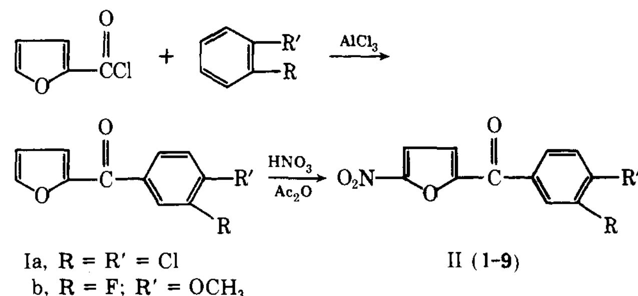
In the course of work directed at the synthesis of furans containing a 5-nitro group and a 2 side chain, 5-nitro-2-furyl phenyl ketone (1) was prepared and found to possess antifungal activity. In this paper we describe the synthesis and biological properties of some aryl 5-nitro-2-furyl ketones II and derivatives.

Chemistry. The aryl 5-nitro-2-furyl ketones II were prepared by nitration of the corresponding aryl 2-furyl ketones I with nitric acid and Ac_2O. The position of nitration had been established previously.[1] Compounds I were prepared by Friedel–Crafts reaction from furoyl chloride and appropriately substituted benzenes in the presence of $AlCl_3$. The synthesis of the aryl 2-furyl ketones I which were precursors for **1–5**, **7**, and **8** had been reported previously.[2] Acylation of *o*-dichlorobenzene to prepare Ia occurred para to the halogen as indicated by the nmr spectrum and analogous acylations.[3] The product from the acylation of *o*-fluoroanisole was assigned structure Ib since the position of acylation of analogous reactions had been reported to be para to the methoxy group,[4,5] and oxidative degradation of Ib with $KMnO_4$ gave the known acid, 3-fluoro-4-methoxybenzoic acid.[6,7]

An alternative synthesis of II by reaction of 5-nitro-2-furoyl chloride with substituted benzenes in the presence of $AlCl_3$ failed to give appreciable amounts of pure product with the exception of **1**. Variation of the order of addition of the reactants, the use of $SnCl_4$ and $BF_3 \cdot Et_2O$ as catalyst, and the use of various solvents failed to improve the reaction. The derivatives of II listed in Table I were prepared by standard procedures as described in the Experimental Section.



Biological Activity. All compounds prepared were tested *in vitro* for antifungal activity against *Candida albicans* and *Microsporum canis* by the agar diffusion–cylinder cup method.[8] The data are presented in Table I, and the reference standard nystatin is included for comparison. The ketones were found to possess the most significant activity, compounds **1**, **4**, and **8** being the most active. Formation of the oxime and *O*-methyloxime derivatives (**10–13**) decreased activity, while formation of the other derivatives (**14–18**) caused complete loss of activity at the concentrations tested.

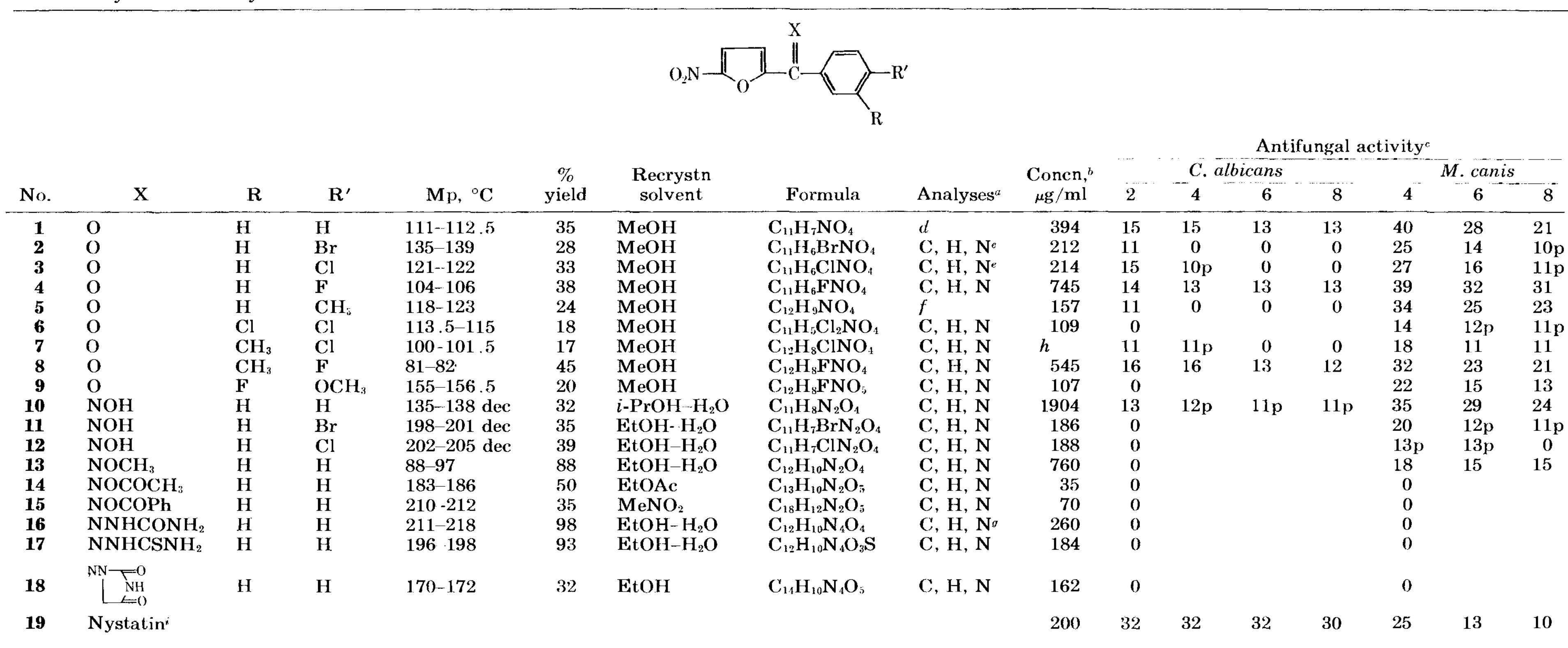
Additional testing of 4 and 8 at 200 μg/ml against *C. albicans* under kinetic fungicidal test conditions showed them to be active, reducing a viable cell count of 3×10^5/ml to 0 after 2 hr; no growth was evident on subsequent subculture after 72 hr.

Experimental Section†

Aryl 5-Nitro-2-furyl Ketones (1–9). Nitric acid (70%, 95 ml, 1.5 mol) containing 2 ml of concentrated H_2SO_4 was added dropwise to 330 ml (3.5 mol) of Ac_2O with stirring and cooling to maintain the temperature at 25–30°. The aryl 2-furyl ketone (0.5 mol) was added in portions over 20 min at 0°. After the mixture was stirred for 0.5 hr, 300 ml of H_2O and 570 g (1.5 mol) of $Na_3PO_4 \cdot 12H_2O$ were added. The mixture was heated with stir-

† Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Boiling points are uncorrected. The ir spectra were determined with a Perkin-Elmer Model 137B spectrophotometer. The nmr spectra were determined with a Varian Associates Model A-60A spectrometer (Me_4Si).

Table I. Aryl 5-Nitro-2-furyl Ketones and Derivatives



No.	X	R	R′	Mp, °C	% yield	Recrystn solvent	Formula	Analyses[a]	Concn,[b] μg/ml	Antifungal activity[c]: *C. albicans* 2	*C. albicans* 4	*C. albicans* 6	*C. albicans* 8	*M. canis* 4	*M. canis* 6	*M. canis* 8
1	O	H	H	111–112.5	35	MeOH	$C_{11}H_7NO_4$	*d*	394	15	15	13	13	40	28	21
2	O	H	Br	135–139	28	MeOH	$C_{11}H_6BrNO_4$	C, H, N[e]	212	11	0	0	0	25	14	10p
3	O	H	Cl	121–122	33	MeOH	$C_{11}H_6ClNO_4$	C, H, N[e]	214	15	10p	0	0	27	16	11p
4	O	H	F	104–106	38	MeOH	$C_{11}H_6FNO_4$	C, H, N	745	14	13	13	13	39	32	31
5	O	H	CH_3	118–123	24	MeOH	$C_{12}H_9NO_4$	*f*	157	11	0	0	0	34	25	23
6	O	Cl	Cl	113.5–115	18	MeOH	$C_{11}H_5Cl_2NO_4$	C, H, N	109	0				14	12p	11p
7	O	CH_3	Cl	100–101.5	17	MeOH	$C_{12}H_8ClNO_4$	C, H, N	*h*	11	11p	0	0	18	11	11
8	O	CH_3	F	81–82	45	MeOH	$C_{12}H_8FNO_4$	C, H, N	545	16	16	13	12	32	23	21
9	O	F	OCH_3	155–156.5	20	MeOH	$C_{12}H_8FNO_5$	C, H, N	107	0				22	15	13
10	NOH	H	H	135–138 dec	32	*i*-PrOH–H_2O	$C_{11}H_8N_2O_4$	C, H, N	1904	13	12p	11p	11p	35	29	24
11	NOH	H	Br	198–201 dec	35	EtOH–H_2O	$C_{11}H_7BrN_2O_4$	C, H, N	186	0				20	12p	11p
12	NOH	H	Cl	202–205 dec	39	EtOH–H_2O	$C_{11}H_7ClN_2O_4$	C, H, N	188	0				13p	13p	0
13	$NOCH_3$	H	H	88–97	88	EtOH–H_2O	$C_{12}H_{10}N_2O_4$	C, H, N	760	0				18	15	15
14	$NOCOCH_3$	H	H	183–186	50	EtOAc	$C_{13}H_{10}N_2O_5$	C, H, N	35	0				0		
15	NOCOPh	H	H	210–212	35	$MeNO_2$	$C_{18}H_{12}N_2O_5$	C, H, N	70	0				0		
16	$NNHCONH_2$	H	H	211–218	98	EtOH–H_2O	$C_{12}H_{10}N_4O_4$	C, H, N[g]	260	0				0		
17	$NNHCSNH_2$	H	H	196–198	93	EtOH–H_2O	$C_{12}H_{10}N_4O_3S$	C, H, N	184	0				0		
18	NN—=O, NH, =O	H	H	170–172	32	EtOH	$C_{14}H_{10}N_4O_5$	C, H, N	162	0				0		
19	Nystatin[i]								200	32	32	32	30	25	13	10

[a]See footnote †. [b]Concentration determined spectrophotometrically. Compounds dissolved in 50% aqueous EtOH. [c]Diameter of zones of inhibition in millimeters on days indicated. [d]Lit. mp 111°; see ref 1, p 153. [e]See H. Saikachi and T. Kitagawa, *Yakugaku Zasshi*, **89**, 1626 (1969); *Chem. Abstr.*, **72**, 43313h (1970). [f]Lit. mp 122–123°; see ref 1, p 153. [g]See F. Zajdela, R. Royer, E. Bisagni, and A. Ennuyer, *Acta Unio Int. Contra Cancrum*, **20**, 233 (1964); *Chem. Abstr.*, **61**, 8784b (1964). [h]Concentration not determined but <2000 μg/ml. [i]Potency = 5100 units/mg.

ring at 50° for 1 hr and cooled in an ice bath. The solid was collected by filtration, washed with H_2O, and recrystallized. See Table I.

3,4-Dichlorophenyl 2-Furyl Ketone (Ia). 2-Furoyl chloride (195 g, 1.5 mol) was added dropwise to a mixture of 200 g (1.5 mol) of anhydrous $AlCl_3$ and 500 ml of ethylene chloride over 15 min with stirring. *o*-Dichlorobenzene (220 g, 1.5 mol) was added dropwise over 20 min. The mixture was heated on a steam bath for 20 hr and poured into a mixture of concentrated HCl and ice. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with 5% $NaHCO_3$ solution and H_2O and dried (K_2CO_3). The solvent was removed by distillation under reduced pressure, and the residual oil was distilled to give 51 g (15%) of Ia: bp 182° (1.0 mm); ir (Nujol) 1640 cm^{-1} (C=O); nmr ($CDCl_3$) δ 6.54 (dd, 1, J = 1.8 and 3.5 Hz, 4-furyl proton), 7.24 (d, 1, J = 3.8 Hz, 3-furyl proton), 7.49 (d, 1, J = 8.2 Hz, 5-phenyl proton), 7.66 (dd, 1, J = 0.9 and 1.7 Hz, 5-furyl proton), 7.81 (dd, 1, J = 2.0 and 8.5 Hz, 6-phenyl proton), and 8.06 ppm (d, 1, J = 2.0 Hz, 2-phenyl proton). *Anal.* ($C_{11}H_6Cl_2O_2$) C, H.

3-Fluoro-4-methoxyphenyl 2-Furyl Ketone (Ib). Anhydrous $AlCl_3$ (117 g, 0.88 mol) was added in portions to a mixture of 104 g (0.80 mol) of 2-furoyl chloride, 100 g (0.80 mol) of *o*-fluoroanisole, and 500 ml of CS_2 over 1.5 hr with stirring and cooling. The mixture was stirred for 3.5 hr at room temperature, heated under reflux for 2.5 hr, allowed to stand overnight, and poured into a mixture of ice and H_2O. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed twice with 10% Na_2CO_3 solution and once with H_2O and dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure, and the residual solid was recrystallized from MeOH to give 148 g (84%) of Ib. Recrystallization again from MeOH gave an analytical sample: mp 80.5-81°; ir (Nujol) 1645 cm^{-1} (C=O). *Anal.* ($C_{12}H_9FO_3$) C, H.

3-Fluoro-4-methoxybenzoic Acid. A solution of 27 g of $KMnO_4$ in 250 ml of hot H_2O was added slowly to a suspension of 13 g (0.06 mol) of Ib in 100 ml of hot H_2O containing 0.2 g of KOH. The mixture was heated on a steam bath for 45 min, and the MnO_2 was removed by filtration. The filtrate was made acidic with concentrated H_2SO_4, and the white solid was collected by filtration to give 3 g (30%), mp 212-214° (lit.[7] mp 211.6-212.8°).

Aryl 5-Nitro-2-furyl Ketone Oximes (10-12). A mixture of 0.25 mol of 1, 2, or 3, 1.0 mol of $NH_2OH \cdot HCl$, 1.0 mol of anhydrous NaOAc, and 2 l. of 75% aqueous EtOH was heated under reflux for 5 hr and cooled. The solid was collected by filtration and dried. See Table I for yields. Analytical samples were prepared by recrystallization from the solvents indicated in Table I.

5-Nitro-2-furyl Phenyl Ketone *O*-Acetyloxime (14). A mixture of 71 g (0.30 mol) of 10, 61 ml (0.60 mol) of Ac_2O, and 700 ml of *p*-dioxane was heated under reflux for 19 hr. After cooling, the mixture was poured into 2 l. of cold H_2O, and the solid was collected by filtration. Recrystallization from EtOAc gave 41 g of 14.

5-Nitro-2-furyl Phenyl Ketone *O*-Benzoyloxime (15). Benzoyl chloride (1.8 g, 0.013 mol) was added dropwise to a solution of 2.3 g (0.01 mol) of 10 in 15 ml of pyridine with stirring. The solution was stirred at room temperature for 6 hr and allowed to stand overnight. Water (30 ml) was added, and the solid was collected by filtration and washed with H_2O. Recrystallization from $MeNO_2$ twice gave 1.2 g of 15.

5-Nitro-2-furyl Phenyl Ketone *O*-Methyloxime (13), Semicarbazone (16), Thiosemicarbazone (17), and Aminohydantoin (18). Concentrated HCl was added dropwise to a hot solution (0.20 mol) of 1 and (0.24 mol) of methoxyamine hydrochloride, semicarbazide hydrochloride, thiosemicarbazide, or 1-aminohydantoin in 600 ml of 80% aqueous EtOH to adjust the pH to 3. The solution was heated under reflux for 4 hr and cooled in an ice bath. The solid was collected by filtration and washed with cold 80% aqueous EtOH. See Table I for yields. Analytical samples were prepared by recrystallization from the solvents indicated in Table I.

Kinetic Fungicidal Test. Hog gastric mucin (Wilson) dissolved in Sabouraud's liquid medium (BBL) at a 5% concentration (w/v) was sterilized by autoclaving. To the cooled medium, sufficient sterile bovine serum was added to obtain a 10% concentration, v/v. Test compounds were dissolved in *N,N*-dimethylacetamide (0.2-0.5 ml), and sufficient medium was added to obtain the requisite test concentrations. The exact amount of solvent used was added to a separate flask for control.

The 24-hr agar slants of the respective yeast strain were harvested in saline. Cell concentrations were determined by a haemocytometer count or Klett-Summerson colorimeter readings plus previously prepared standard growth curves. Sufficient cells were added to the test medium to give 4×10^5 cells/ml. Aliquots were obtained from the flask samples, serially diluted in saline, and plated in medium to obtain a viable count. Samples were taken at 0, 2, 4, 7, 24, and 72 hr. The data obtained would indicate whether the test compound was active, inactive, fungistatic, or fungicidal and the rate of fungicidal activity if present.

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Synthesis of Pyrimido[4,5-*b*][1,4]-7-hydrothiazines Related to 7,8-Dihydropteridines of Biological Importance†,‡

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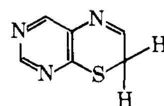
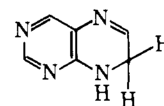
7,8-Dihydrofolic acid and related 7,8-dihydropteridines are key metabolic intermediates in a variety of biological reactions, while antagonists of such compounds have been found to have useful antineoplastic[1,2] and immunosuppressant activities.[3] It has been reported that substituted 7,8-dihydropteridine analogs of antagonists of folic and pteroic acid may be more potent inhibitors of *Streptococcus faecalis* and of *Pediococcus cerevisiae* than their nonreduced pteridine analogs.[4,5]

Since most of the useful biological properties of substituted pteridines are related to their abilities to form dihydro and tetrahydro derivatives, it is unfortunate that the high reactivity of these reduced forms both to oxidative degradation and photoreactions makes it difficult to work with them.[6] Work with reduced analogs of folic acid, pteroic acid, and related derivatives is also complicated by the extremely low solubilities of these compounds at physiological pH, presumably due to hydrogen bonds formed by their ring nitrogens. It was hoped that replacement of the 8-nitrogen of reduced pteridines with sulfur would provide compounds with greater stabilities and better solubility characteristics than those of their analogous 7,8-dihydropteridine derivatives. While several members of this ring system have been prepared previously,[7-12] none of these have been related to biologically active dihydropteridines.



pyrimido[4,5-*b*][1,4]-7-hydrothiazine



7,8-dihydropteridine

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