were determined at the Merck Sharp & Dohme Research Laboratories by Mr. Jack Gilbert and his associates. Where analyses are indicated only by symbols of elements, they are within ±0.4% of the theoretical values. Proton NMR spectra were recorded on a Varian Model T-60 spectrometer using tetramethylsilane as internal standard. ^{13}C NMR spectra were obtained with a Varian CFT-20 spectrometer.

General Methods. Method A. 1-(4-Chlorophenyl)-4-methoxypyridinium Fluorosulfonate (24). A solution of 18.7 g (0.091 mol) of carefully dried 1-(4-chlorophenyl)-4(1*H*)-pyridinone (**23**)[2] in 160 mL of dimethoxyethane was heated to reflux. Without further external heating, 14.2 g (0.123 mol) of methyl fluorosulfonate was added gradually. The mixture boiled vigorously during the addition and crystalline product started to separate. After the addition was complete, the mixture was stirred at room temperature for 1 h and then cooled to 10 °C. The product was filtered, washed with cold dimethoxyethane and Et_2O, and dried in vacuo: yield 28.0 g (96.5%); mp 182–185 °C; NMR (Me_2SO-d_6) δ 4.26 (s, 3 H, CH_3O), 6.27 (d, 2 H, β-pyridyl H), 6.32 (s, 4 H, aromatics), 7.62 (d, 2 H, α-pyridyl H). Anal. C, H, N, Cl.

Method B. 1-(4-Chlorophenyl)-4(1*H*)-pyridinone Hydrazone Fluorosulfonate (2). To a suspension of 3.2 g (0.01 mol) of **24** in MeOH (20 mL) was added 0.4 g of anhydrous hydrazine. The mixture was heated to boiling for a few minutes and the resulting clear orange solution was kept overnight at room temperature. The orange crystals of **2** obtained in two crops weighed 2.97 g (93%). This product was recrystallized from MeOH (Table I).

Method C. 4-Chlorobenzaldehyde 1-(4-Chlorophenyl)-4(1*H*)-pyridinylidene Hydrazone Fluorosulfonate (4). To a solution of 2.4 g (7.5 mmol) of **2** in EtOH (35 mL) and H_2O (30 mL) was added a solution of 1.27 g (9 mmol) of *p*-chlorobenzaldehyde in EtOH (5 mL). After heating the reaction mixture on the steam bath for 3 min, the mixture was concentrated to remove most of the EtOH, cooled, and filtered: yield 3.1 g (94%). Recrystallization from EtOH–H_2O gave mp 252–253 °C (Table I).

Method D. Conversion of Compound 4 to Free Base 4a. A sample of **4** was partitioned between CH_2Cl_2 and 2 N NaOH. The organic layer was concentrated to dryness and the residue recrystallized from EtOH–H_2O: mp 165–166 °C (Table I).

Method E. Direct Formation of 4a. To a solution of 2.4 g (7.5 mmol) of **2** in 15 ml of 0.5 N NaOEt in EtOH at 30 °C was added 1.27 g (9 mmol) of *p*-chlorobenzaldehyde. After stirring at 30–35 °C for 0.5 h the solution was refrigerated. The yield of **4a** in two crops was 2.3 g (90%) which was recrystallized from EtOH–H_2O (Table I).

Method F. 4-Chlorobenzaldehyde 1-Methyl-4(1*H*)-pyridinylidene Hydrazone (20). A mixture of 2.31 g (0.01 mol) of 1-[(4-chlorophenyl)methylene]-2-(4-pyridyl)hydrazine (**19**), 2.5 g (0.018 mol) of CH_3I, and EtOH (20 mL) was refluxed for 20 h. On cooling 3.2 g of **20**·HI (86%) was obtained. The salt was converted to free base **20** by method D (Table II).

Acknowledgment. The computer program used to superimpose the structures discussed in this paper was developed by J. D. Andose and M. Pensack. Determinations of pK_a values were by Dr. George B. Smith. Dr. Graham Smith performed some of the molecular modeling calculations.

Supplementary Material Available: A listing of atomic coordinates used for structures 4 and **25** (4 pages). Ordering information is given on any current masthead page.

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Hydrophobicity of Several Rhodium(II) Carboxylates Correlated with Their Biologic Activity[1]

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Rhodium(II) carboxylates differ greatly in antitumor activity and toxicity depending on the properties of the carboxylate group (methoxyacetate, propionate, butyrate, etc.) involved. The solubility characteristics of rhodium(II) carboxylates correlate well with both the antitumor activity and toxicity that these compounds display. The amount of rhodium which is adsorbed by tumor cells in vitro also correlates with the partition coefficient of the rhodium(II) compounds studied. Survival and toxicity studies show rhodium(II) pentanoate to possess the highest therapeutic index against the Ehrlich ascites tumor strain and also show that lengthening the carboxylate R chain beyond the pentanoate reduces the drugs' therapeutic efficacy.

Recently, our laboratories reported on the antitumor activity of some selected rhodium(II) carboxylates (Figure 1).[2,3] Survival studies on Swiss mice bearing the Ehrlich ascites tumor showed that the series, rhodium(II) acetate and rhodium(II) propionate, exhibited increasing toxicity and antitumor activity when compared on a millimoles per kilogram basis.[4] An earlier study showed that rhodium(II) acetate in combination with arabinosylcytosine gave significant increases in survival times and 50-day "cures" of BDF_1 mice bearing the L1210 ascites tumor.[5] Since rhodium(II) acetate and propionate differ only by the length of the carbon chain, yet vary significantly in antitumor activity and toxicity, it seemed reasonable to hypothesis that an increase in lipophilicity might correlate with an increase in antitumor activity. In the study reported here, this hypothesis was tested using a number of

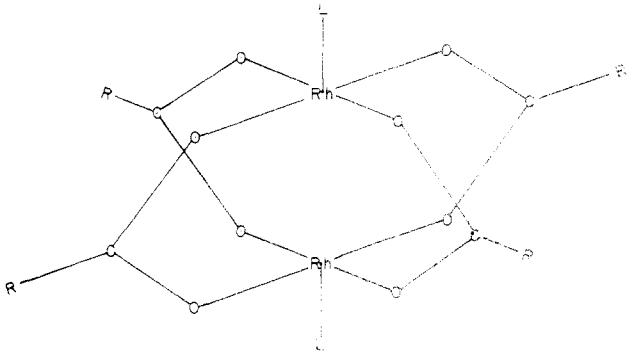

Figure 1. The structure of rhodium(II) carboxylates: L, axial ligands; R, carbon chain of the carboxylate groups; R = $-CH_3$, rhodium(II) acetate; R = $-CH_2CH_3$, rhodium(II) propionate; R = $-CH_2CH_2CH_3$, rhodium(II) butyrate; R = $-CH_2(CH_2)_2CH_3$, rhodium(II) pentanoate; R = $-CH_2(CH_2)_3CH_3$, rhodium(II) hexanoate; R = $-CH_2OCH_3$, rhodium(II) methoxyacetate.

rhodium(II) carboxylates. The results showed that a correlation exists between the hydrophobicity of the rhodium(II) carboxylate and antitumor activity and toxicity. The results also indicated that absorption of rhodium(II) carboxylates by cells is governed primarily by the compounds' hydrophobic character.

Experimental Section

Materials and Methods. Chemicals. Rhodium(II) acetate and rhodium(III) chloride were purchased from Matthey Bishop, Inc., Malvern, Pa. 19355. Rhodium(II) methoxyacetate, rhodium(II) propionate, and rhodium(II) butyrate were synthesized from the acetate precursor by exchange with the free acid.[6] Rhodium(II) pentanoate and rhodium(II) hexanoate were synthesized by an oxidation–reduction reaction previously described.[7]

Survival Studies. Female Swiss albino mice (Sprague-Dawley, Madison, Wis. 53711) (20–25 g) were each implanted ip with 6×10^6 Ehrlich tumor cells. Twenty-four hours after implantation, all mice were randomly divided into groups of ten. Rhodium(II) methoxyacetate, acetate, and propionate were dissolved in 0.9% saline with gentle heating when necessary. Due to the insolubility of rhodium(II) butyrate in saline, this compound was initially dissolved in 95% ethanol and then, just prior to injection, diluted with 0.9% saline to a final concentration of 3% ethanol. The other rhodium(II) carboxylates, rhodium(II) pentanoate and rhodium(II) hexanoate, were found to precipitate immediately from the ethanol upon addition of saline solution. For this reason, an alternate carrier, isopropyl myristate, had to be used. In order to correlate the results from the two different carriers, the propionate and butyrate analogues were also studied in isopropyl myristate. Rhodium(II) acetate was found to be only partially soluble in isopropyl myristate and would not yield reliable doses. All rhodium(II) carboxylate solutions were prepared fresh each day just prior to injection. Drug treatment was started 24 h after tumor implantation and injections were given ip once daily for 6 days. Control groups received ip injections of either saline, saline plus ethanol, or isopropyl myristate once daily for 6 days. Observations were continued for 50 days.

Measurement of LD_{10} of Rhodium(II) Carboxylates. The method of Skipper et al.[8] was employed. Briefly, five different groups of ten healthy female Swiss albino mice per group received ip five different doses of rhodium(II) carboxylate. Nine days later plots of mortality vs. dose were made for each drug. The LD_{10} for each rhodium(II) complex was then determined from the corresponding graph. This dose was then administered to ten mice to test the validity of the extrapolated dosage.

Determination of Partition Coefficients. Determination of chloroform–saline partition coefficients for the seven rhodium(II) compounds was carried out as follows. A known amount of anhydrous rhodium(II) carboxylate was dissolved in 0.9% saline solution buffered by 0.05 M phosphate (pH 7.4) and the molar absorptivity determined (a_{MAc} = 227.3, a_{Ac} = 220.5, a_{Prop} = 221.6; at λ_{max} 586 nm). Rhodium(II) butyrate, pentanoate, and hexanoate were so insoluble in the saline–buffer solution that it was necessary to determine their absorptivities in the chloroform solution (a_{But} = 228.2, a_{Pent} = 205.3, a_{Hex} = 210.0; at λ_{max} 615 nm). The concentration of rhodium(III) chloride in the aqueous layer was determined by atomic absorption at 3435 Å.

The saline buffer solution (100 mL) which contained the predominantly water-soluble rhodium compound was shaken with an equal volume of chloroform until equilibrium was established. The concentration of the rhodium compound was then determined in the appropriate layer. The chloroform solution of the chloroform soluble rhodium(II) carboxylates was equilibrated in an equal volume of saline–buffer in like manner.

Determination of 1-octanol–saline partition coefficients was carried out in the manner described above. Rhodium concentrations, however, were determined in both the 1-octanol and saline–buffer by atomic absorption spectroscopy at 3435 Å.

Measurement of Rhodium Uptake by Ehrlich Tumor Cells. The method of atomic absorption spectroscopy was used to measure the amount of rhodium firmly bound to Ehrlich ascites tumor cells that had been incubated with the different rhodium compounds. Tumor cells were removed from the abdominal cavity of Swiss mice by gentle aspiration with a Pasteur pipet. The collected cells were diluted with 8.0 mL of 0.9% saline and centrifuged at 1470g for 2 min in an IEC internation clinical centrifuge. The supernatant was decanted and the remaining packed cells were stirred for 30 s with 4.0 mL of iced distilled water to lyse the red blood cells, diluted with 4.0 mL of 0.8% saline, and sedimented for 2 min at 1470g. This procedure was repeated and the cell concentration determined with a Coulter counter. The cells were washed with 5 vol of Fischer bicarbonate buffer[9] and finally diluted 1:2 with Fischer tissue medium[9] (pH 7.8; 1×10^8 cells/mL) containing the designated rhodium compound. The final concentration of all the rhodium compounds, except $Rh_2(Prop)_4$, was 0.1 mM. The final concentration of $Rh_2(Prop)_4$ was 0.05 mM due to the insolubility of this compound. The cell suspensions were incubated with shaking in air for 2 h at 37 °C in a precision Scientific Dubnoff metabolic shaking incubator. After incubation, cells were transfered to glass conical centrifuge tubes and a 1-mL aliquot of Fischer medium was used to rinse the 25-mL incubation flask. Rinses were added to appropriate tubes and the cells were pelleted at 1470g for 2 min. Supernatants were removed and the cells were rewashed twice (Fischer bicarbonate buffer), then finally diluted 1:5 with iced distilled water, and stored at 20 °C prior to rhodium analysis.

The measurements for rhodium content were made with a Perkin-Elmer Atomic Absorbance Spectrophotometer Model 303 using a rhodium cathode lamp at the 3434-Å resonance line, 30-mA current, and an air–acetylene flame with air and gas flow of 8 psi each. The detection limit of the instrument was observed to be 0.3 ppm of rhodium.

Analytical controls were prepared by adding known aliquots of the rhodium stock solutions to 1:5 aqueous suspensions of tumor cells or buffer, and the percent recovery of the rhodium was determined by atomic absorption spectroscopy.

As a check on the homogeneity and viability of the tumor population, an aliquot of untreated and $Rh_2(Ac)_4$-treated tumor cells incubated as described above was sedimented and washed twice with 0.9% saline, and the packed tumor cells were diluted 1:5 with 0.9% saline. Aliquots (0.2 mL) of the two cell suspensions (6×10^6 cells) were injected into the ip cavities of two groups of mice containing three healthy mice each and their survival times determined.

As an additional check on the homogeneity of the tumor cells in the suspension, untreated and rhodium(II) acetate treated tumor cells were examined after incubation for 2 h at 37 °C in Fischer tissue medium, as described above, by electron microscopy. Primary fixation of the tumor cells was done with glutaraldehyde in cacodylate buffer (pH 7.4) according to the method of Dawes.[10] Postfixation of the cells was performed in 1.0% osmium tetraoxide in cacodylate buffer (pH 7.4) and the cells were then embedded in Spure's resin. After trimming and sectioning, the cells were stained with uranyl acetate and examined with a Hitachi electron microscope.

Results

Partition Coefficients. The partition coefficients of the rhodium(II) carboxylates and $RhCl_3$ are summarized

Table I

Compd	Saline LD_{10} (mol/kg × 6 days)	Saline % ILS[b]	Saline TI	Isopropyl myristate LD_{10} (mol/kg × 6 days)	Isopropyl myristate % ILS	Isopropyl myristate TI	% Rh bound to tumor cells	Partition coeff[a] Chloroform	Partition coeff[a] 1-Octanol
$RhCl_3$	4.2×10^{-3}			Insoluble			12.0	<0.0005	1.32×10^{-4}
$Rh_2[OOCCH_2OCH_3]_4$	5.9×10^{-4}	50	1.1	Insoluble			2.5	<0.005	5.21×10^{-2}
$Rh_2[OOCCH_3]_4$	2.6×10^{-4}	88	1.8	Insoluble			25.0	0.071	6.54×10^{-2}
$Rh_2[OOCCH_2CH_3]_4$	1.9×10^{-5}	149	4.2	1.2×10^{-5}	175	2.0	68.0	0.540	8.17
$Rh_2[OOC(CH_2)_2CH_3]_4$	6.0×10^{-6}	197	4.7	1.4×10^{-6}	179	2.4	Insoluble	15.6	898
$Rh_2[OOC(CH_2)_3CH_3]_4$	Insoluble			1.7×10^{-6}	236	3.1	Insoluble	>300	>3000
$Rh_2[OOC(CH_2)_4CH_3]_4$	Insoluble			2.2×10^{-6}	136	2.0	Insoluble	>300	>3000

[a] All partition coefficients were determined at 37 °C. [b] % ILS and TI are based on the LD_{10} dose.

in Table I. Rh_2MAc_4 and $RhCl_3$ were found to have limited solubility in the chloroform layer, causing the partition coefficients to approach zero for both Rh_2MAc_4 and $RhCl_3$, within the errors of these experiments. The partition coefficients of the rhodium(II) complexes of acetic, propionic, and butyric acid were 0.071, 0.543, and 15.6, respectively. As the carbon chain was increased beyond the butyrate, to the pentanoate and hexanoate derivatives, the rhodium(II) carboxylates were found, within the detection limit of the spectrometer, to be insoluble in the aqueous layer.

Results similar to the chloroform experiment were obtained using 1-octanol as the lipid layer. The 1-octanol–saline partition coefficients of $RhCl_3$ were again found to be small, 1.32×10^{-4}. Both Rh_2MAc_4 and Rh_2Ac_4 were found to be predominantly soluble in the saline–buffer with partition coefficients of 5.21×10^{-2} and 6.54×10^{-2}, respectively. Rhodium(II) propionate, with a partition coefficient of 48.17, showed only intermediate solubility while Rh_2But_4, with a partition coefficient of 898, was predominantly soluble in the lipid layer. Neither the pentanoate nor hexanoate derivatives could be detected (limit of detection was 50 ppm) in the aqueous layer. The lower limit of their partition coefficient was determined to be 3000.

Survival and Toxicity Studies. The condensed results of survival studies employing the Ehrlich ascites tumor in female Swiss mice are shown in Table I. The LD_{10} for the rhodium(II) carboxylates studied in saline and isopropyl myristate also appear in Table I. The results show that the antitumor activity and toxicity increase as the hydrophobic carbon chain of the carboxylate is extended to the pentanoate, followed by a decrease in antitumor activity and toxicity as the chain continues to be lengthened.

Rhodium Uptake by Tumor Cells. The results, which are summarized in Table I, indicate significant differences in the absorption by the tumor cells of the four rhodium compounds used in this study. Control experiments, where known aliquots of the rhodium compounds were added to 1:5 aqueous suspensions of tumor cells, showed that 85% of the rhodium present in the sample was detected by atomic absorption spectroscopy. When known aliquots of the rhodium stock solution were added to Fischer tissue medium or Fischer bicarbonate buffer, the detection of rhodium was quantitative. The lessened detection of the rhodium in the cell samples was probably due to the increased viscosity of the sample, which would retard the aspiration rate of the solution into the flame of the instrument.

The average survival time of the three mice implanted with 6×10^6 tumor cells, which had previously been incubated with the Fischer tissue medium only, was 11 ± 2 days. Two of the mice implanted with the rhodium acetate treated tumor cells lived 21 and 22 days, respectively, with one 50-day survivor. The uptake of the rhodium acetate by the tumor cell population during incubation is clearly indicated by the increased survival time.

The results of examination of the cells by electron microscopy indicated a homogeneous cell population with an average nucleus size of 8.5 u and an average cell size of 12.3 u. No consistent difference could be detected in the electron micrographs of the rhodium acetate treated cells when the prints made from these cells were compared with those obtained from the untreated tumor cells.

Discussion

Rhodium(II) carboxylates represent a new class of antitumor agents, distinctly different chemically from the platinum complexes. The stable cage structure of dimeric rhodium(II) lends itself to variation in the complexed carboxylate and, therefore, invites study as to which factors have predominant roles in the observed biologic activity. As the R chain of the rhodium(II) carboxylate is altered to make a more lipid-soluble compound and, therefore, increase the partition coefficient, a number of observations can be made.

The fact that the solubility of rhodium(II) carboxylates is determined by the solubility characteristics of the complexed carboxylate ion is not surprising. The two rhodium(II) cations bridged by four monocarboxylic acid anions yield a neutral compound. This lack of charge tends to decrease the water solubility of all the rhodium(II) compounds. Solubility arises, primarily, from solvent interactions at the two axial positions. In the case of rhodium(II) acetate and methoxyacetate no interference of the axial bonding with solvent occurs; hence, these rhodium compounds favor water over chloroform since the axial interactions with the solvent are greater in water. As hydrophobic R groups are incorporated on the rhodium(II) carboxylates, the axial ligand positions tend to desolvate.[11,12] Once axial interactions with solvent have been decreased the rhodium(II) carboxylates, in keeping with the lack of charge and nonpolar characteristics of these compounds, tend to dissolve in less polar solvents.

As the lipophilicity (partition coefficient) is increased antitumor activity and toxicity also increase. Figure 2 shows the correlation between the observed toxicity and the partition coefficient for five of the seven rhodium compounds tested. Linear least-squares regression of the data, shown in Figure 2, yielded a regression coefficient of 0.9848. This correlation suggests that increased amounts of the drug are absorbed by cells as the drug becomes more lipid soluble. Measurements of the amount of rhodium absorbed by tumor cells (Table I) show this to be true. Rhodium(II) propionate, which has the highest partition coefficient of the four compounds tested in the absorption experiment, also is absorbed the most by tumor cells in vitro. In addition, the more lipid-soluble rhodium com-

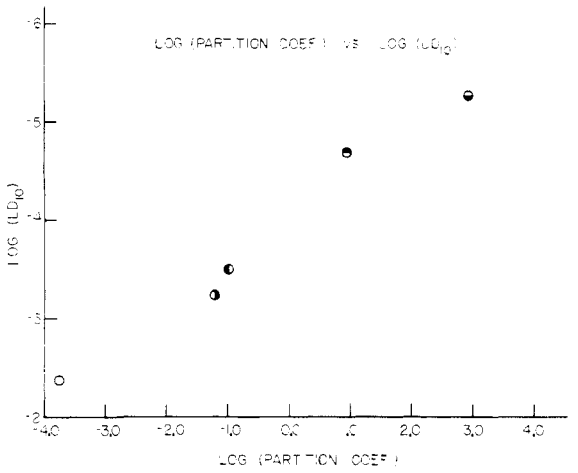

Figure 2. Correlation between the 1-octanol–saline partition coefficients and the LD_{10} of rhodium(II) carboxylates: ○, rhodium(II) chloride; ◑, rhodium(II) methoxyacetate; ◐, rhodium(II) acetate; ◓, rhodium(II) propionate; ◒, rhodium(II) butyrate.

pounds would have greater ability to dissolve into proteins, such as enzymes, and exert a biological effect.[13]

The amount of rhodium absorbed by tumor cells increases as the partition coefficient increased. The antitumor activity and therapeutic indices also increased through rhodium(II) pentanoate. The observed decrease in antitumor activity, toxicity, and therapeutic efficacy of the hexanoate complex suggests that either steric problems are beginning to be seen or rhodium(II) compounds must exhibit some intermediate degree of water solubility to be effective.

This study shows that rhodium(II) carboxylates present promise as antitumor drugs in the treatment of neoplasias. However, the simple extension of the carboxylate R chain beyond the butyrate or pentanoate is not effective in altering the therapeutic possibilities of the drug. Because of the lipid solubility of rhodium(II) complexes the study of their effect against CNS neoplasias would be merited. Also, the synthesis of other rhodium(II) carboxylates with functional R groups may prove to be advantageous.

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(4) Abbreviations used: ILS, increase in life span; TI, therapeutic index; a, molar absorptivity; ip, intraperitoneal; LD_{10}, that dose that kills 10% of an animal population from drug toxicity; Cl, chloride; MAc, methoxyacetate; Ac, acetate; Prop, propionate; But, butyrate; Pent, pentanoate; Hex, hexanoate; rhodium(II) acetate, tetra-μ-acetato-dirhodium(II); rhodium(II) propionate, tetra-μ-propionato-dirhodium(II); rhodium(II) butyrate, tetra-μ-butyrato-dirhodium(II); rhodium(II) pentanoate, tetra-μ-pentanoato-dirhodium(II); rhodium(II) hexanoate, tetra-μ-hexanoato-dirhodium(II); rhodium(II) methoxyacetate, tetrakis-μ-methoxyacetato-dirhodium(II).
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Antiprotozoal Thiazoles. 2. 2-(5-Nitro-2-furyl-, thiazolyl-, and 1-methylimidazolyl-)thiazoles

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Ten 2-substituted 4-thiazolecarboxaldehyde hydrazones bearing 5-nitro-2-furyl, 5-nitro-2-thiazolyl, and 1-methyl-5-nitro-2-imidazolyl functions have been prepared and screened for activity against *Trypanosoma cruzi* infections in mice. The results permitted the ranking of these substituents in decreasing order of activity: 1-methyl-5-nitro-2-imidazolyl > 5-nitro-2-furyl > 5-nitro-2-thiazolyl, the last being inactive. Some structural features of the side chain necessary for optimum activity are discussed. The most active compound, 4-[[[2-(1-methyl-5-nitro-2-imidazolyl)-4-thiazolyl]methylene]amino]thiomorpholine 1,1-dioxide, compared favorably with the standard Nifurtimox against three recent clinical isolates of *T. cruzi*, including one with a high myocardial tissue infiltration.

A recent report[1] from these laboratories has described the synthesis and antitrypanosomal activity of a series of 2-(5-nitro-2-thienyl)-4-thiazolecarboxaldehyde hydrazones. The purpose of this second paper is to report the extension of this work to three other nitro heterocyclic nuclei, i.e., furan, thiazole, and 1-methylimidazole together with a comparison of their activity.

Chemistry. The syntheses of these compounds required the corresponding 5-nitro-2-cyano heterocycles as intermediates. 5-Nitro-2-furancarbonitrile[2] and 5-nitro-2-thiazolecarbonitrile[3] were prepared by published procedures. 1-Methyl-5-nitro-2-imidazolecarbonitrile[4] was readily prepared in high yield from the carboxaldehyde by treatment with *O,N*-bis(trifluoroacetyl)hydroxylamine.[5] The conversion of these nitriles to the thiocarboxamides **1–3** proceeded smoothly by the Taylor–Zoltewicz method[1,6] in good yield (Table I). Subsequent Hantzsch cyclization with 1,3-dichloroacetone was carried out as described previously to give the 4-chloromethylthiazoles **4–6** (Table II). Compounds **5** and **6** were converted to the 4-