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Structure and Antischistosomal Activity in the Nitrofurylvinyl and the Niridazole Series. Noninterchangeability of the Nitroheterocyclic Rings†

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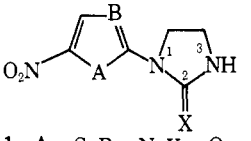
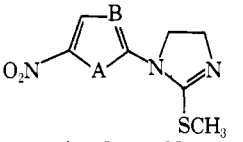
Good antischistosomal activity is shown by nitrofurylvinyl derivatives such as amides of 3-(5-nitro-2-furyl)acrylic acid (**4b** and **5b**), as well as by nitrothiazole derivatives such as niridazole (**1a**). The effects of interchanging the nitroheterocyclic groupings have been studied by the synthesis and biological comparison of five pairs of exact analogs. Replacement of nitrofuran by nitrothiazole in the nitrofurylacrylic acid amides (**4b** and **5b**) gave **4a** and **5a** and resulted in complete loss of antischistosomal activity. Substitution of nitrothiazole by nitrofuran in niridazole (**1a**) and two new active analogs **2a** and **3a** gave the exact analogs **1b**, **2b**, and **3b**, respectively, with essentially complete loss of activity. These findings are surprising in view of the close similarity of biochemical and morphological effects produced by compounds of the nitrofurylvinyl and of the niridazole series. Comparisons of partition coefficients and of nitro group oxidation potentials suggest that these factors alone cannot explain all the data, and it is suggested that subtle structural differences as well as differences in metabolism are also involved.

In previous studies[2,3] we have determined structural features which appear to be essential for antischistosomal activity of 5-nitro-2-furyl derivatives. These features comprise a nitrofuran linked *via* an olefinic bond to a terminal nitrogen substituent of low basicity. However, a nitrothiazole derivative (niridazole, **1a**) is also prominent among the relatively few nitroheterocyclic derivatives which show good schistosomicidal activity. We have already pointed out[2] that nitrofuran derivatives, such as *trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole (**6**, SQ 18,506) and amides of 3-(5-nitro-2-furyl)acrylic acid (*e.g.*, **4b** and **5b**), as well as the nitrothiazole **1a** exhibit the same time course and pattern of biochemical and morphological changes in schistosomes, suggesting a common mode of action. Furthermore, in spite of the dissimilar side chains borne by the nitroheterocyclic rings, comparison of models revealed striking similarities. Specifically, superimposition of the nitroheterocyclic moieties of **6** or **4b** *vs.* **1a** resulted in reasonable overlap not only of the respective terminal side-chain nitrogen substituents but also of the vinyl group of **6** or **4b** with the N_1–C_2 bond of **1a**.[4] These conclusions rested on assumptions about the preferred conformations of the compounds under discussion. These assumptions have recently been supported by X-ray crystallographic studies‡,§ with **1a** and **6**.

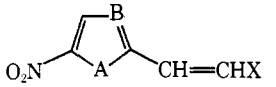
The question now arose as to the possible interchangeability of the nitroheterocyclic groupings in the nitrofurylvinyl and niridazole series. It is already known that in the nitrofurylvinyl series replacement of nitrofuran by nitrophenyl results in complete loss of activity,[4] while replacement by nitrothienyl has an adverse effect.[5] Furthermore, in the niridazole series, replacement of nitrothiazole by



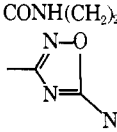
1a, A = S; B = N; X = O
b, A = O; B = CH; X = O
2a, A = S; B = N; X = S
b, A = O; B = CH; X = S



3a, A = S; B = N
b, A = O; B = CH



4a, A = S; B = N; X = $CONHCH(CH_3)_2$
b, A = O; B = CH; X = $CONHCH(CH_3)_2$
5a, A = S; B = N; X = $CONH(CH_2)_2$-c-NC_5H_{10}·HCl
b, A = O; B = CH, X = $CONH(CH_2)_2$-c-NC_5H_{10}·HCl



6, A = O; B = CH; X =

nitrophenyl and nitropyridyl similarly results in complete loss of activity.# However, no data have hitherto been available for direct comparison of the biologically effective nitrofuran and nitrothiazole groupings. Consequently, the

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‡ R. T. Puckett and B. Biffar, Ciba-Geigy Corp., unpublished data.

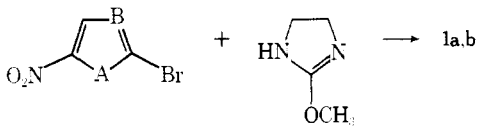
§ L. Amzel, Johns Hopkins University, personal communication.

D. W. Henry and E. Bueding, unpublished data.

synthesis and testing of exactly analogous pairs of nitrofuran and nitrothiazole derivatives were undertaken. Compounds **1b**, **2a,b**, **3a,b**, **4a**, and **5a** were prepared and these, taken together with the known compounds **1a**, **4b**, and **5b**, afforded five exactly corresponding pairs for biological evaluation. In the nitrothiazolylvinyl series, the acrylic amide derivatives rather than aminooxadiazole derivatives were synthesized because of greater synthetic accessibility.

Chemistry. We attempted to synthesize 1-(5-nitro-2-furyl)-2-imidazolidinone (**1b**), the nitrofuryl analog of niridazole, *via* reaction of 5-nitro-2-furyl isocyanate (generated *in situ* by Curtius rearrangement of the corresponding azide) with 2-chloroethylamine or ethanolamine. These reactions failed to give the expected ureas. We therefore turned to 2-bromo-5-nitrofuran (**8**), which is known[6] to undergo reaction at C-2 with nucleophiles such as morpholine and other secondary amines. However, **1b** was not obtained when **8** was treated with 2-imidazolidone anion, generated *in situ* by the reaction of imidazolidinone with $NaNH_2$. Instead, intractable tarry mixtures resulted, presumably because of the base sensitivity of nitrofurans.

We then took advantage of the known[7] nucleophilicity of 2-methoxy-2-imidazoline (**9**) and found that 2-bromo-5-nitrofuran (**8**) when treated with 2 molar equiv of **9** in DMF at 80° for 4 hr gave **1b** in 16% yield. The yield was not improved by changing solvents (DMSO, MeOH) or by varying the molar quantity of **9**. At lower temperature (<60°), a thermally unstable product, tentatively formulated as 2-methoxy-1-(5-nitro-2-furyl)-2-imidazoline, was also obtained, whereas at 80° or higher temperature only **1b** was isolated. This type of reaction between a bromonitro heterocyclic compound and 2-methoxy-2-imidazoline (**9**) may offer a general route to imidazolidone-substituted nitro heterocyclic products. Indeed, when the readily available 2-bromo-5-nitrothiazole (**7**) was treated with **9** in DMF at room temperature, niridazole **1a** was readily obtained in 17% yield (no attempt was made to optimize the yield). This represents a simple new synthetic route to this clinically useful drug. The urea carbonyl groups of **1a** and **1b** show infrared absorptions at 1750 and 1775 cm^{-1} (KBr), respectively, which are markedly higher than the

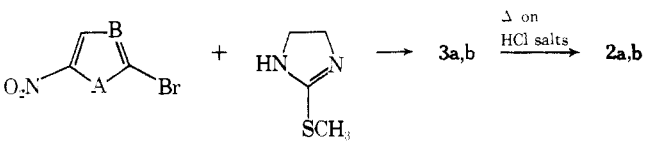


7. A = S; B = N
8. A = O; B = CH

9

1a,b

1680 cm^{-1} observed for unsubstituted 2-imidazolidinone. These substantial shifts of the carbonyl absorptions are attributed to the strong electron-withdrawing effect of the 5-nitro heterocyclic systems.



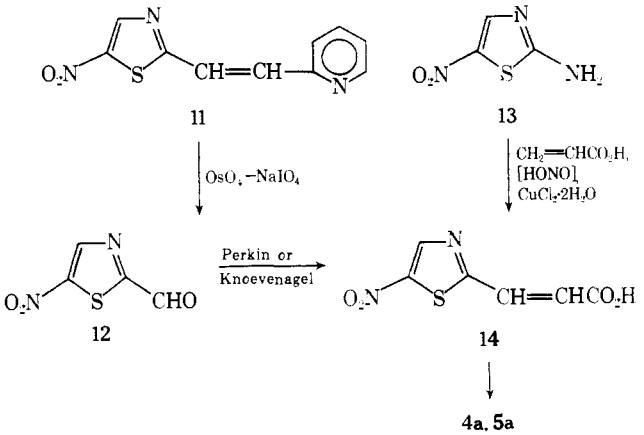
7. A = S; B = N
8. A = O; B = CH

10

3a,b —Δ on HCl salts→ **2a,b**

Treatment of **7** and **8** with 2-methylmercapto-2-imidazoline (**10**) in DMF at 80° and room temperature, respectively, gave the corresponding nitro heterocyclic pseudothioureas **3a,b** as the sole products. While our work was in progress, the preparation of the former compound by a different synthetic route was described.[8] We were also able to convert **3a** to **1a** by the use of H_2O_2–AcOH at 70°. The corresponding conversion of **3b** to **1b** could be accomplished at room temperature by the same reagents, while at 70° the reaction led only to decomposition products. These reactions provide another convenient method to obtain niridazole **1a** and its furan analog **1b**. The pseudothioureas **3a,b** readily give HCl salts, which on heating cleaved to the thioureas **2a,b**.

In our first synthetic approach to the nitrothiazoleacrylic acid amides **4a** and **5a**, the known[9] 2-(5-nitro-2-thiazolyl)vinylpyridine (**11**) was oxidatively cleaved by the OsO_4–$NaIO_4$ method to give 5-nitrothiazole-2-carboxaldehyde (**12**).[10] Reaction of **12** with acetic anhydride or malonic acid under Perkin or Knoevenagel conditions, respectively, gave the desired 3-(5-nitro-2-thiazolyl)acrylic acid (**14**) in poor yield (3% in both cases), due to decomposition of the nitrothiazole system by the bases (NaOAc or pyridine) used in these reactions. We therefore sought a more effective route to **14**, and, indeed, when 2-amino-5-nitrothiazole (**13**) was treated with acrylic acid under Meerwein arylation[11] conditions, the desired acid **14** could be obtained in improved yield (15%) in one step. The acid **14** was then converted into the *N*-isopropylamide **4a** and the *N*-[2-(1-piperidinyl)ethyl]amide (as HCl salt, **5a**) by the mixed anhydride method.

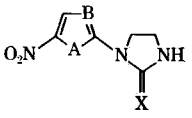


Pharmacological Results. The compounds were tested for antischistosomal activity against *Schistosoma mansoni* according to our reported procedures,[2,4] except that the compounds were suspended in an emulsifying agent "Cremophor EL" (BASF) (concentration, 25%). Using this modification, the antischistosomal activity of these and other nitroheterocyclic compounds was increased to an extent of approximately 40%. The pharmacological results are shown in Table I. While compounds **1a** (niridazole) and **2a** possess pronounced antischistosomal properties, their furyl analogs **1b** and **2b** show little effect on the worms. Replacement of the thiazole ring by furan in niridazole greatly increases the host toxicity. Compound **3a** also exhibits significant schistosomicidal activity, but it is very toxic at curative dose levels; its furan analog **3b** is even more toxic and is also inactive.

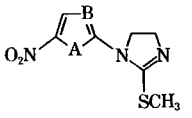
At a dose level of 125 mg/kg twice a day for 3 days, compounds **2a** and **3a** produce identical short-term effects. However, **3a** has more lasting effects, showing a greater reduction in the number of worms and higher parasitological cures than does **2a**.

Among the nitrofurylacrylic acid amide series, **4b** and **5b** have the highest activity,[2] causing significant biological and morphological changes in the worms. However, their thiazole analogs, **4a** and **5a**, respectively, are completely devoid of these antischistosomal properties.

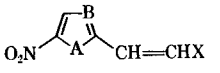
Table I. Short-Term and Long-Term Antischistosomal Effects of 5-Nitro-2-furyl and 5-Nitro-2-thiazolyl Derivatives on *S. mansoni* in Mice



1a,b, 2a,b



3a,b



4a,b, 5a,b, 6

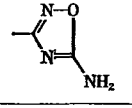
Compd	A	B	X	Dosage schedule	Mouse mortality	Short-term effects, 3 days after last dose: Reduction of phosphorylase phosphatase, %	Reduction of glycogen levels, %	Damage to reproductive system, %	Hepatic shift, %	Long-term effects, 4–5 weeks after last dose: Reduction in no. of worms, %	Parasitological cures, %
1a	S	N	O	Oral: 250 mg/kg b.i.d., 6 doses	12	72	68	78	90	100	100
				75 mg/kg b.i.d., 8 doses	0	94	88	87	100	98	60
1b	O	CH	O	Oral: 125 mg/kg b.i.d., 2 doses, + 83 mg/kg b.i.d., 4 doses	30	14	19	10	0	0	0
				75 mg/kg b.i.d., 4 doses	30	0	0	0	0	0	0
				Iv: 35 mg/kg, 2 doses on 2 consecutive days	30	22	18	14	0	0	0
				Im: 25 mg/kg, 2 doses on 2 consecutive days	25	28	25	22	0	0	0
				25 mg/kg, 1 dose	0	0	0	27	0	0	0
2a	S	N	S	Oral: 250 mg/kg b.i.d., 6 doses	25	64	59	58	50	96	50
				125 mg/kg b.i.d., 6 doses	10	50	47	67	20	50	0
				Im: 200 mg/kg, 2 doses at 2-day intervals	25	37	33	46	10	17	0
				200 mg/kg, 1 dose	0	11	8	61	0	0	0
2b	O	CH	S	Oral: 125 mg/kg b.i.d., 6 doses	12	14	16	31	0	8	0
3a	S	N		Oral: 250 mg/kg b.i.d., 5 doses	65	81	76	67	80	97	67
				125 mg/kg b.i.d., 6 doses	25	52	47	67	55	87	30
				55 mg/kg b.i.d., 8 doses	0	48	43	78	78	60	0
				Im: 200 mg/kg, 2 doses	25	30	24	25	0	0	0
				200 mg/kg, 1 dose	0	0	0	42	0	0	0
3b	O	CH		Oral: 250 mg/kg, 1 dose, + 62.5 mg/kg, 3 doses, + 31.25 mg/kg, 1 dose	80	8	12	17	0	0	0
				55 mg/kg b.i.d., 8 doses	30	4	0	14	14	6	0
				Iv: 100 mg/kg, 1 dose	25	8	12	47	0	0	0
4a	S	N	$CONHCH(CH_3)_2$	Oral: 250 mg/kg b.i.d., 6 doses	0	0	0	7	0	0	0
4b	O	CH	$CONHCH(CH_3)_2$	Oral: 250 mg/kg b.i.d., 6 doses	0	100	100	100	100	83	14
5a	S	N	$CONH(CH_2)_2$-c-NC_5H_{10}·HCl	Oral: 250 mg/kg b.i.d., 6 doses	10	0	8	17	0	0	0
5b	O	CH	$CONH(CH_2)_2$-c-NC_5H_{10}·HCl	Oral: 250 mg/kg b.i.d., 6 doses	10	83	87	38	90	91	20
6	O	CH		Oral: 160 mg/kg, 3 doses at 12-hr intervals	0	100	94	78	100	100	100

Table II. Octanol–Water Partition Coefficients[a] and Oxidation Potentials[b] of 5-Nitro-2-furyl and 5-Nitro-2-thiazolyl Derivatives

Compd	Log P	E_h, mV
1a	0.91	−360
1b	0.25	−400
2a	1.70	
2b	0.98	
3a	1.78	−365
3b	1.19	−410
4a	1.76	−260
4b	1.34	−255
6	1.30	

[a] The partition coefficients were determined according to the procedures of Fujita, *et al.*,[12] except that the concentrations of the compounds in the octanol layer were determined by uv measurement. [b] These determinations were made using a Sargent Model 21 polarograph and a saturated calomel reference electrode. Measurements were carried out at room temperature, and the compounds were dissolved in mixtures of dimethylformamide and borax–potassium diphosphate buffer of pH 7.4 exactly according to the procedure of Kutter, *et al.*[5]

Discussion

The pharmacological results demonstrate that replacement of the nitrothiazole moiety of niridazole by nitrofuran essentially destroys antischistosomal activity. Conversely, in the nitrofurylvinyl series substitution of nitrofuran by nitrothiazole results in complete loss of activity. This indicates remarkable structural specificity for these nitro heterocyclic derivatives and raises the question as to which factors are involved. Thus, the lipophilic character of the compounds, their oxidation potentials, and subtle differences in their structural and conformational features may be of particular importance.

To test the role of lipophilicity, we measured the octanol-water partition coefficients[12] of the compounds. The experimental results (Table II) suggest that log P values of 0.9-1.3 are best for antischistosomal activity:** *i.e.*, compound **1a**, 0.91; **6**, 1.30; **4b**, 1.34. These values are comparable to the value of 0.91 found for optimum lipophilicity in an unrelated series of antischistosomal compounds.[13] Interestingly, we have found that the value of compound **1b** (0.25) falls well below this optimum range. This might explain its lack of activity. However, lipophilic character alone cannot explain the pharmacological results manifested by some of the other nitro heterocycles. Thus, whereas compounds **2b** and **3b** possess partition characteristics similar to the active compounds **1a**, **6**, and **4b**, they are devoid of antischistosomal activity. On the other hand, compounds **2a** and **3a** (both showing significant antischistosomal activity) and **4a** (inactive) all show comparable lipophilic character which is above the optimum range. Clearly, other factors determine biological activity in these cases.

For some of these 5-nitro heterocyclic compounds we have observed substantial differences between the $\Sigma\pi$ values (obtained from additive calculations using functional partition constants[14]) and the experimental log P values. Although log P may be calculated in many instances using partition constants no good data of this kind appear to exist for these systems.

We have demonstrated the importance of the nitro group for antischistosomal activity in the nitrofurylvinyl series and have suggested[3] that *in vivo* reduction of the nitro group (either by the host or the parasite) is involved. Correlations have been made between oxidation potential and biological activity for several antibacterial and antiprotozoal nitro heterocyclic derivatives,[8,15] while reduction of the nitro group of niridazole in rat liver systems is known.[16] One may therefore seek to correlate the antischistosomal activity of the compounds reported in this paper with their oxidation potentials. Niridazole (**1a**) and compound **3a** possess higher oxidation potentials than their furan analogs **1b** and **3b** by 40 and 45 mV, respectively†† (Table II), and **1a** and **3a** are therefore likely to be more easily reduced *in vivo* (by "nitro reductase"). If the postulate is valid that antischistosomal activity is directly related to *in vivo* reduction of the nitro group, these results would be consistent with the difference in biological activity between these nitrothiazole and nitrofuran analogs. Similar correlations have been made for nitrobenzofurans,[17] in which active antibacterial compounds were found to possess higher oxidation potentials than inactive compounds (ΔE_h 80 mV). However, since the oxidation potential of active nitrofuryl compound **4b** is similar to that of the inactive thiazole analog **4a**, the biological activity of these particular vinyl compounds is clearly determined by other factors. We have already noted that **4b** and **4a** differ in lipophilic character (log P 1.34 and 1.76, respectively) but since the log P value for the inactive compound **4a** is close to that of the active niridazole analogs **2a** and **3a** (Table II), this lipophilicity difference between **4b** and **4a** may not be important.

Subtle differences in bond lengths and bond angles might also have significant effects on biological activity and might indeed explain the difference between **4b** and **4a**. A detailed X-ray crystallographic study of niridazole (**1a**) has been carried out by Puckett and Biffar.‡ The study shows that the two rings are essentially coplanar and that there is significant delocalization of the N_1 p electrons with the thiazole ring, as we had postulated earlier.[4] In addition, an X-ray crystallographic study of compound **6** is in progress.§ It is already clear that the molecule is planar and that the side-chain double bond is transoid with respect both to the 2,3 double bond of the furan and the oxadiazole C=N bond. However, the structure refinement does not yet provide precise bond length and bond angle data. We expect to obtain these data in the near future so that direct comparison of structural and conformational features between the nitrofuran and nitrothiazole derivatives can be made. Additionally, metabolism of these compounds may also play an important role in determining their biological activity. We plan to investigate this question.

It is of interest that the thio analogs (**2a** and **3a**) of niridazole (**1a**) both show significant antischistosomal activity. However, they are each less active and more toxic than niridazole. It is not known if the activity is intrinsic or is due to *in vivo* conversion to niridazole.

In conclusion, the studies reported in this paper show that replacement of nitrofuran by nitrothiazole in the nitrofurylvinyl series results in complete loss of antischistosomal activity. Conversely, replacement of nitrothiazole by nitrofuran in the niridazole series results in essentially complete loss of activity. These results are unexpected if the two series indeed have the same biochemical mode of action. Our studies also show that differences in lipophilicity or in the oxidation potential of the nitro group might explain some of these results. However, other factors such as differences in metabolism and subtle structural differences are also likely to be important.

** A detailed and comprehensive study of the relationship between lipophilicity and antischistosomal activity for nitro heterocyclics is planned in collaboration with Dr. M. Cory, Stanford Research Institute.

†† The oxidation potentials were determined by Dr. I. Weiner, Upstate Medical Center, SUNY, Syracuse, N. Y.

Experimental Section

All compounds were identified by nmr, ir, and mass spectroscopy. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. Melting points were determined on a Kofler hot-stage melting point apparatus and are uncorrected.

1-(5-Nitro-2-furyl)-2-imidazolidinone (Ib). Method A. 2-Bromo-5-nitrofuran (8,[6] 5.76 g, 30 mmol) and 2-methoxy-2-imidazoline (9,[18] 6 g, 60 mmol) were dissolved in DMF (60 ml) and the mixture was heated at 80° for 4 hr. DMF was then removed under reduced pressure and the residue was refluxed with EtOAc. The mixture was filtered and the filtrate evaporated. The resulting residue was triturated with anhydrous ether and the insoluble product was chromatographed on silica gel (EtOAc eluent) giving unreacted 2-bromo-5-nitrofuran (3.84 g), followed by compound 1b. Recrystallization from dioxane-benzene gave 315 mg (16% yield) of 1b, mp 238–239°. *Anal.* ($C_7H_7N_3O_4$) C, H, N.

Method B. Compound **3b** (50 mg, 22 mmol) in glacial AcOH (4 ml) and 30% H_2O_2 (200 mg) in glacial AcOH (4 ml) were stirred at room temperature for 4 hr. H_2O (20 ml) was added; the solution was made slightly basic using 5% $NaHCO_3$ and extracted (EtOAc). The extracted product was column-chromatographed (silica gel) and recrystallized using the same systems as in method A to give pure 1b (26 mg, 60%).

1-(5-Nitro-2-thiazolyl)-2-imidazolidinone (Niridazole, 1a). 2-Bromo-5-nitrothiazole (7, 1.045 g, 5 mmol) and compound 9 (1 g, 10 mmol) were dissolved in DMF (15 ml) and the solution was stirred at room temperature for 8 hr. DMF was removed under reduced pressure and the residue was chromatographed on silica gel (EtOAc eluent), giving starting material 7 (115 mg) and then compound 1a (162 mg, 17%). The ir and nmr of the product were identical with those of an authentic sample of 1a as were the melting point and mixture melting point.

1-(5-Nitro-2-thiazolyl)-2-methylthio-2-imidazoline (3a). 2-Bromo-5-nitrothiazole (7, 3.72 g, 13 mmol) and 2-methylmercapto-2-imidazoline (10, 3 g, 26 mmol) were dissolved in DMF (30 ml) and the mixture was stirred at room temperature for 20 hr. The precipitated crystals were recrystallized from acetone to give 3a (1.035 g, 33% yield), mp 189–190° (lit.[8] mp 195–197°). *Anal.* ($C_7H_8N_4O_2S_2$) C, H, N, S.

1-(5-Nitro-2-furyl)-2-methylthio-2-imidazoline (3b). 2-Bromo-5-nitrofuran (8, 3.7 g, 19.2 mmol) and compound 10 (4.45 g, 38.3 mmol) in DMF (40 ml) were stirred at 80° for 8 hr. DMF was removed under reduced pressure and the residue refluxed with EtOAc. The EtOAc solution was separated from insoluble material and was evaporated. The residue was column-chromatographed (EtOAc-silica gel) to give starting material 8 (1.32 g) and then compound 3b. The latter was recrystallized from acetone to give 405 mg (14% yield) of 3b, mp 188–189°. *Anal.* ($C_8H_9N_3O_3S$) C, H, N, S.

1-(5-Nitro-2-thiazolyl)-2-imidazolidinethione (2a). 3a (840 mg, 3.44 mmol) was stirred in *i*-PrOH saturated with HCl gas (30 ml) at room temperature for 2 hr. Ether (200 ml) was added and the analytically pure HCl salt of 3a precipitated (963 mg, 100% yield); mp 172–175°. *Anal.* ($C_7H_9ClN_4O_2S_2$) C, H, Cl, N, S. The HCl salt was heated at 180° for 1.5 hr to give pure 2a (791 mg, 100% yield), mp 257–258° dec. *Anal.* ($C_6H_6N_4O_2S_2$) C, H, N, S.

1-(5-Nitro-2-furyl)-2-imidazolidinethione (2b). The HCl salt of 3b was prepared in quantitative yield in the same manner as described for the HCl salt of 3a and had mp 159–161°. *Anal.* ($C_8H_{10}ClN_3O_3S$) C, H, Cl, N, S. The product (263 mg, 1 mmol) was heated at 160° for 30 min and the resulting solid was dissolved in DMF (5 ml) and chromatographed on silica gel (EtOAc eluent) to give analytically pure 2b (132 mg, 62% yield), mp 190–193°. *Anal.* ($C_7H_7N_3O_3S$) C, H, N, S.

3-(5-Nitro-2-thiazolyl)acrylic Acid (14). Method A. From 5-Nitrothiazole-2-carboxaldehyde (12). Compound 12, which was obtained from the OsO_4-$NaIO_4$ oxidation of 11 in a modification of Henry's method,[10] was treated with $CH_2(CO_2H)_2$-pyridine[19] or with Ac_2O-$NaOAc$[20] to give 14 in about 3% yield.

Method B. Meerwein Arylation Reaction. From 2-Amino-5-nitrothiazole (13). To a mixture of concentrated HCl (55 ml) and water (13 ml), 17.4 g (0.12 mol) of 13 was added and the slurry was cooled to about −30°. To this mixture, 9 g (0.13 mol) of $NaNO_2$ in 15 ml of water was introduced slowly. The mixture was stirred for 10 min and freshly distilled acrylic acid (13 g, 0.18 mol) in acetone (75 ml) was added while the temperature was kept at −30°. $CuCl_2 \cdot 2H_2O$ (3.5 g) was then added and the mixture stirred for 2 hr at −30° before it was allowed to rise to room temperature. At −20°, evolution of N_2 was vigorous and the mixture became reddish. After cessation of nitrogen evolution, water (200 ml) was added and the mixture extracted with EtOAc. The organic phase was washed with 5% $NaHCO_3$, and the water layer was made acidic (10% HCl) and extracted with EtOAc. The extract was then washed (H_2O), dried, and evaporated to give the product (14), which was recrystallized from EtOAc-$CHCl_3$ (1:1) (3.5 g, 15% yield); mp 207–208°. *Anal.* ($C_6H_4N_2O_4S$) C, H, N, S.

***N*-Isopropyl-3-(5-nitro-2-thiazolyl)acrylamide (4a).** 14 (2 g, 10 mmol) in 30 ml of EtOAc was cooled to −70°. To this solution, 1.01 g (10 mmol) of *N*-methylmorpholine in 10 ml of EtOAc was added, followed by 1.09 g (10 mmol) of redistilled ethyl chloroformate. After the mixture was stirred at −70° for 30 min, 650 mg (11 mmol) of isopropylamine in 20 ml of EtOAc was added, and the mixture stirred for 1 hr. A 3% HCl solution (50 ml) was added while the reaction mixture was allowed to reach room temperature. The EtOAc portion was then washed (3% $NaHCO_3$, saturated NaCl), dried (Na_2SO_4), and evaporated. The resulting product was dissolved in acetone and filtered through a short column of alumina. Evaporation of the solvent gave 4a, which was recrystallized from EtOAc-$CHCl_3$ (1:1) (1.4 g, 58% yield). The crystals transformed at 165–170° into needles, mp 189–190°. *Anal.* ($C_9H_{11}N_3O_3S$) C, H, N, S.

***N*-[2-(1-Piperidinyl)ethyl]-3-(5-nitro-2-thiazolyl)acrylamide (5a).** To a solution of the acid 14 (2g, 10 mmol) in EtOAc (30 ml) which was cooled to −30°, *N*-methylmorpholine (1.1 g, 11 mmol) in EtOAc (10 ml) was added, followed by a solution of redistilled ethyl chloroformate (1.09 g, 10 mmol) in 10 ml of EtOAc. The mixture was stirred for 15 min at −30° and filtered. While maintaining the temperature at −30°, a solution of EtOAc (10 ml) containing 2-(1-piperidinyl)ethylamine (1.28 g, 10 mmol) was added dropwise and the reaction mixture stirred for 30 min. Dry HCl gas was then passed in and the product 5a precipitated. After flushing away excess HCl gas with N_2, the product was collected and was recrystallized from EtOH-C_6H_6 (2 g, 58%), mp >345°. *Anal.* ($C_{13}H_{19}ClN_4O_3S$) C, H, Cl, N, S.

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Synthesis of Alkyl-Substituted α,β-Unsaturated γ-Lactones as Potential Antitumor Agents

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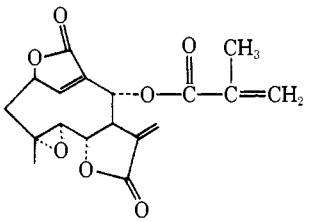
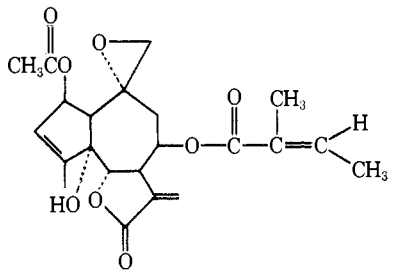
A series of alkyl-substituted di- and monolactones including (*E,E*)-3,3′-(alkanediylidene)bis[dihydro-2(3*H*)-furanones] **9–12** and the monolactones **18** and **19** was synthesized by reaction of α-(triphenylphosphoranylidene)-γ-butyrolactone with appropriate aldehydes. The reaction of this ylide with formaldehyde gave α-methylene-γ-butyrolactone (**20**). These compounds were tested for antitumor activity as part of a study to determine the influence of β substituents and distance between alkylating sites on the antitumor activity of α,β-unsaturated lactones. The testing was carried out in standard NCI screens and the compounds possessed ED_{50} values of 16–110 μg/ml against cells derived from human carcinoma of the nasopharynx (KB) and were inactive against L1210 lymphoid leukemia in the mouse.

Extensive screening of plant extracts has led to the isolation of a large number of sesquiterpene lactones having cytotoxic and/or antitumor activity.[1-4] Those compounds which possess *in vivo* antitumor activity are characterized by their polyfunctionality as evidenced by examination of the structures of elephantopin (**1**),[5] euparotin acetate (**2**),[6] and vernolepin (**3**).[7] The importance of the α-methylene-



1



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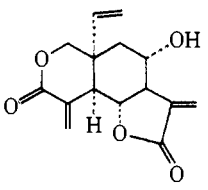

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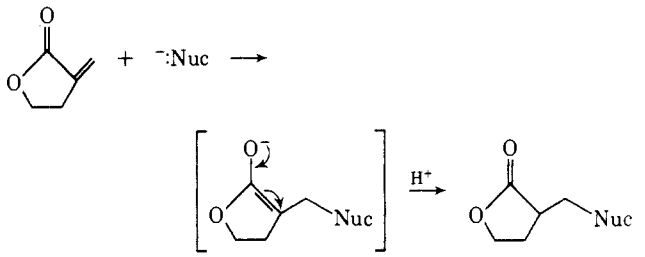
γ-lactone moiety to the activity of these and related compounds has been demonstrated in a series of studies by Kupchan and coworkers.[1] For example, saturation of the conjugated double bond in the α-methylene-γ-lactone groups of vernolepin and elephantopin results in loss of cytotoxic activity. The α-methylene-γ-lactone group appears to act as an alkylating agent by virtue of the Michael addition of biological nucleophiles across the conjugated double bond as depicted in Scheme I.[8]

The usefulness of the natural sesquiterpene lactones has been limited by their relatively high toxicity. In the natural compounds the β-carbon (alkylating site) of the α-methylene lactone system is unsubstituted. A substituent at this position should produce changes in physical properties and in chemical reactivity which could alter biological activity and therefore have important biological implications. In support of this concept Semonsky and coworkers[9] have demonstrated that variation of substituents at the α and β carbons of γ-crotonolactones has significant effects on antitumor activity. We have therefore undertaken the synthesis of a variety of substituted as well as unsubstituted α-methylene-γ-lactones in an attempt to systematically vary the electronic and steric environment at the β position of the conjugated lactone. Because of the apparent relationship between polyfunctionality and *in vivo* antitumor activity, the synthesis of difunctional compounds has been emphasized. The monolactones reported were designed to serve as model compounds and as precursors to mixed difunctional alkylating agents. The utility of mixed difunctionality among certain alkylating agents has recently been demonstrated.[10]

Scheme I



Synthesis. To explore the effect of alkyl substitution on antitumor activity, α,β-unsaturated dilactones **9–12** were synthesized (Scheme II). The range of distances between potential alkylating sites in this series encompasses those found between potential alkylating sites in all of the known natural sesquiterpene lactone tumor inhibitors. The general approach to the preparation of these and related compounds involved the Wittig reaction between ylide **4** and the appropriate aldehyde. The preparation of this ylide[11] and its reaction with aromatic aldehydes to give substituted γ-lactones[12] have been reported. This route appeared to offer easy extension to a variety of alkyl-substituted γ-lactones under relatively mild reaction conditions.

The aldehyde precursors to lactones **9–12** included 1,5-