(7) L. B. Kier, *J. Pharm. Sci.*, **61**, 1394 (1972).
(8) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).
(9) M. Bodanszky, *Nature (London)*, **175**, 685 (1955).
(10) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **88**, 1338 (1966).

Chemotherapeutic Nitroheterocycles. Antischistosomal Properties of Nitrofurylvinyl and Nitrothienylvinyl Heterocycles

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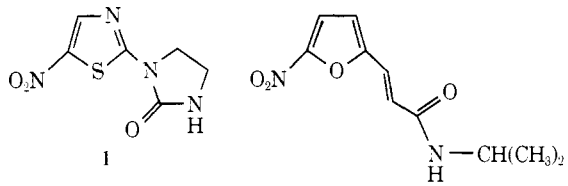
A series of 24 analogs of the experimental antischistosomal agent, 5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole (**3**), was prepared and evaluated in mice infected with *Schistosoma mansoni*. Although antischistosomal activity was widespread in the series, only four of the compounds showed significant curative properties. Compounds containing 2-imidazolyl (**4**) and 2-pyridyl (**15**) groups gave cure rates around 25% at 400 and 250 mg/kg dose levels, respectively. The 2-thiazolyl (**8**) and 2-pyrimidyl (**18**) derivatives were especially notable, yielding 100% cures at 250 and 200 mg/kg dose levels, respectively.

Schistosomiasis has proven to be one of the most intractable diseases to which man is subject, and as yet no practical and generally effective drugs or methods of control are available.[1,2] Among the many types of drugs that have shown potentially useful antischistosomal activity, the nitroheterocycles have demonstrated unusual promise. Niridazole (**1**) has achieved some clinical use in the treatment of infections with *Schistosoma haematobium* and *Schistosoma mansoni*.[3] Furapromidium (**2**) has been reported to be effective in limited trials against *Schistosoma japonicum*.[1,4,5] More recently, Robinson, *et al.*,[6-9] found another experimental nitrofuran, 5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole (**3**, SQ 18506), that possesses high activity against *S. mansoni* and *S. japonicum* in mice, hamsters, and monkeys as well as lower host toxicity compared with **1**, **2**, and other related compounds. As part of this study, these authors noted that activity in this class of compounds could be correlated with certain common structural features. One of the required features is a 5-nitrofuran or 5-nitrothiazole nucleus linked through the 2 position to a rigid side chain bearing a low-basicity nitrogen. The nitrogen atom must be located in a specific spatial position relative to the nitroheterocyclic nucleus. In **1** and **2**, the nitrogen atom of the NH moiety in the urea and the amide groups, respectively, fills this role; and in **3**, one of the oxadiazole ring nitrogens occupies the same position.



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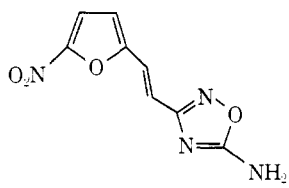


3

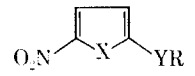
Further development of the hypothesis by Hulbert, *et al.*,[7] has led to the conclusion that the nature of the side chain connecting the key nitrogen atom to the nitroheterocyclic nucleus is critical. Working with the furanacrylamide system of furapromidium, they found that altering the geometry and/or electronic properties of the system by replacing the vinyl bridge with ethylene or acetylene linkages totally eliminated antischistosomal activity. On the other hand, a variety of acrylamides with varying substituents on the amide nitrogen retained the desired biochemical and chemotherapeutic properties.

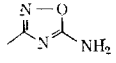
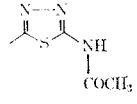
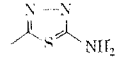
Further studies aimed at delineating the limits of the initial structure–activity hypothesis[6] are reported in this paper. Specifically, we have examined the effect of altering the heterocyclic ring system through which the low-basicity nitrogen is introduced into analogs of **3**. In addition, we have examined the antischistosomal properties of the 5-nitro-2-thienyl group as a replacement for the nitrofuran moiety in some of these analogs. Using a carboxamide moiety to replace the vinyl bridge of active compounds was also evaluated.

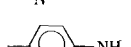
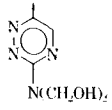
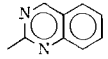
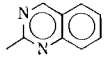
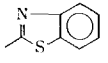
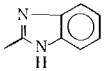
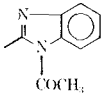
Chemistry. The 5-nitrofuran-2-vinyl and 5-nitrothiophene-2-vinyl compounds in Table I were prepared by condensation of 5-nitrofurfural or 5-nitrothiophene-2-carboxaldehyde with appropriate methyl-substituted heterocycles in acetic anhydride–acetic acid solvent (method A). For the imidazole (**4** and **5**) and benzimidazole (**26**) compounds, the *N*-acetyl derivatives (**6**, **7**, and **27**) were isolated as intermediates and subsequently hydrolyzed by 6 *N* HCl (method B). The carboxamide analogs **12**, **14**, and **19** were prepared by reaction of 5-nitro-2-furoic acid chloride and the appropriate amino heterocycle (method C). As noted in Table I, several of the compounds were known previously, usually for their antibacterial properties.

Pharmacology. The antischistosomal activity of this group of compounds is characterized by a consistent course of biochemical and morphological events.[6,7,10] Within 2 days following the administration of an active compound to mice infected with *S. mansoni*, the activity of glycogen phosphorylase phosphatase, of the worms, is reduced, along with an associated decrease in glycogen levels. The biochemical changes are observed at low dose levels of active compounds that do not bring about a shift

Table I



					Short-term effects (3 days after last dose)						Long-term effects (4–5 weeks)						
No.	R	X	Y	Doses	Dose, mg/kg b.i.d.	% mouse mortality	% inhibition of phosphorylase phosphatase	% reduction of glycogen	% damage to female worm reproductive system	% hepatic shift	% reduction of worms	Parasitol cures	Method[a]	Yield, %[b]	Mp, °C	Recrystn solvent	Formula[c]
3		O	CH=CH	5	150	0	90–100	90–100	90–100	90–100	100	100					
4		O	CH=CH	8 10	200 400	0 60	46 78	37 63	71	50 95	21 28	0 25	B	38	223[d]	CH_3CN	
5		S	CH=CH	10	200	20	22	15	17	15	0	0	B	52	245–250	HOAc	$C_9H_7N_3O_2$·HCl·0.25-$C_2H_4O_2$
6		O	CH=CH	10	200	0	34	46		25	26	0	A	43	163–164	EtOAc	$C_{11}H_{11}N_3O_4$
7		S	CH=CH	10	200	0	15	11	13	22	14	0	A	21	185–191	EtOAc	$C_{11}H_{11}N_3O_3S$
8		O	CH=CH	10 10 10	250 250 250	0 10 10	77	68	83	95	100 100 100	100 100 100	A	32	154–156[e]	CH_3CN–H_2O (1:1)	
9		O	CH=CH	10	250	30	61	69	83	90	66	0	A	25	200–201	EtOAc	$C_9H_9N_3O_3S$
10		O	CH=CH	10	200	0	22	11	13	20	8	0	A	48	>300[f]	DMSO–H_2O (1:1)	
11		O	CH=CH	10	200	0	73	80	95	90	86	50	B	65	241–243[g]	DMSO–H_2O (1:1)	
12		O	CONH	10	200	0	8	11	17	0	9	0	C	25	258–259	DMSO–H_2O (1:1)	$C_7H_7N_4O_3S$
13		O	CH=CH	5 6	200 125	100 10	86 52	90 47	76 67	100 60	Toxic 78	Toxic 50	A, B	28	214–216	EtOH	$C_7H_5N_4O_2$

14		O	CONH	10	250	10	0	0	18	10	0	0	C	61	+300	DMSO–H_2O (1:1)	$C_7H_5N_5O_4$
15		O	CH=CH	10	250	0	70	56	97	75	91	20	A	19	177–178[h]	CH_3CN–H_2O	
16		S	CH=CH	10	200	0	21	28	50	25	19	0	A	40	183–185	$CH_3O(CH_2)_2OH$	$C_{11}H_8N_2O_2S$
17		O	CH=CH	10	250	50	0	0	13	0	0	0	A	35	164–165[i]	CH_3CN	
18		O	CH=CH	10 10	200 100	0 0	91 53	86 50	100 93	100 35	100 18	100 0	A	35	220–221[j]	CH_3CN	
19		O	CONH	10	200	0	0	0	7	0	0	0	C	69	210–211	EtOH–H_2O (1:1)	$C_9H_6N_4O_4$
20		O	CH=CH	10	250	0	0	0	0	0	0	0	A	71	218–219[k]	Dioxane	
21[l]		O	CH=CH	10 10 10	100 50 25	90 60 0	83 37	89 30	98 15	70 0	28 14	0 0					
22[m]		O	CH=CH	10 10 10	500 250 75	65 50 0	72 68 47	78 82 40	 89	100 100 35	 64	 29					
23		O	CH=CH	5	250	0	0	0	18	0	0	0	A	39	255–257	Dioxane	$C_{14}H_9N_3O_3$
24		S	CH=CH	10	200	0	20	22	15	11	10	0	A	41	212–213	$CH_3O(CH_2)_2OH$	$C_{14}H_9N_3O_2S$
25		O	CH=CH	10	250	0	0	7	0	0	0	0	A	37	203–206[n]	EtOH–H_2O (1:1)	
26		O	CH=CH	10	250	50	58	74	73	75	Toxic	Toxic	B	63	287 dec[n]	DMF–H_2O (1:1)	
27		O	CH=CH	16	250	25	59	76	80	60	Toxic	Toxic	A	53	175–178[r]	Dioxane	

[a]See Experimental Section for methods. [b]Yield is of analytically pure material and is minimum. [c]Analysis for C, H, and N within 0.4% of theory where empirical formulas are given. [d]Lit. mp 223–225°: A. Fujita, J. Arotomo, S. Minami, and H. Takamatsu, *Yakugaku Zasshi*, **86,** 427 (1966); *Chem. Abstr.*, **65,** 3870*b* (1966). [e]Lit. mp 154–156°: C. Boehringer and G. M. B. H. Soehne, Belgian Patent 630,163 (1964); *Chem. Abstr.*, **61,** 14516*a* (1964). [f]Lit. mp +300°: K. Miura, T. Ohashi, S. Matsuda, and Y. Igarashi, *Yakugaku Zasshi*, **83,** 771 (1963); *Chem. Abstr.*, **59,** 13912*a* (1963). [g]Lit. mp 245°: K. Miura, *Antimicrob. Ag. Chemother.*, 275 (1962); *Chem. Abstr.*, **59,** 13233*b* (1963). [h]Lit. mp 176–177°: K. Harada and S. Emoto, *Chem. Pharm. Bull.*, **13,** 389 (1965). [i]Lit.[f] mp 163–164°. [j]Lit. mp 204–205°: H. Takamatsu, S. Minami, K. Fujimoto, and M. Shimizu, Japan Patent 14,693 (1964); *Chem. Abstr.*, **62,** 572*d* (1964). [k]Lit.[h] mp 223°. [l]Obtained from Boehringer Pharmaceuticals, Mannheim, Germany. [m]Obtained from Fusan Chemical Co., Tokyo, Japan. [n]Lit.[d] mp 202–204°. [o]Lit.[d] mp 174–175°. [p]Lit.[d] mp 293°.

in the location of the worms from the mesenteric veins to the hepatic sinuses. Compounds that, at the highest tolerated doses, caused only these biochemical changes but no hepatic shift were considered to have only low antischistosomal activity. More active compounds, such as niridazole (1) or 3, produced a hepatic shift that was followed by the partial or complete elimination of the worms, as determined 5–6 weeks after drug administration. The chemotherapeutic activity was thus evaluated on the basis of short- and long-term effects (see Table I).

In this and in the previous[6,7] series of nitroheterocyclic compounds, the short-term biochemical changes have proven to be quite a reliable indicator of long-term antischistosomal activity. In only one out of a total of 96 compounds was a reduction in the number of worms detectable after 6 weeks without an initial marked reduction in glycogen phosphorylase phosphatase activities and in glycogen levels of the parasites.

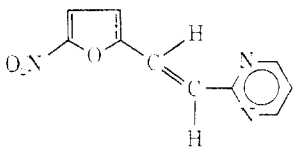
Another initial effect of active compounds was damage to the reproductive system of female schistosomes detectable by an *intra vitam* staining method.[11] However, the predictive value of this criterion proved to be far less reliable. Although administration of every active compound produced more or less pronounced changes in the female reproductive system, some inactive compounds had the same effect, which, in such instances, was reversible.[6]

The determination of glycogen phosphorylase phosphatase activities and of the glycogen levels of the worms, as well as the methodology used for the evaluation of the long-term antischistosomal effects, has been reported elsewhere.[6,8,11,12]

Results and Discussion

With one exception (17), all of the compounds reported in Table I were selected to meet or closely approximate the previously described criteria for antischistosomal activity in nitroheterocycles.[6] The parameter most extensively evaluated in this work is the effect of varying the nature of the carrier of the low-basicity, side-chain nitrogen atom required by the hypothesis. Several, readily available five-membered heterocycles 4–14, six-membered heterocycles 15–22, and bicyclic benzologs 23–27 of active members of the first two groups were used in this evaluation. Antischistosomal activity was widespread in the series, but several new activity-limiting parameters emerged. When the nitroheterocycle was nitrofuran, the bridging moiety (Y of Table I) was vinyl, and the carrier heterocycle was monocyclic (*i.e.*, 4, 6, 8, 9–11, 13, 15, 17, 18, and 20–22); all compounds but two (17 and 20) demonstrated substantial short-term effects and a significant reduction in worm load. However, curative properties were not as widespread. Significant cure rates were produced by 2-imidazolyl (4), 2-thiazolyl (8), 2-amino-5-(1,3,4-thiadiazolyl) (11), 3-(1,2,4-triazolyl) (13), 2-pyridyl (15), and 2-pyrimidyl (18). The 2-thiazolyl (8) and 2-pyrimidyl (18) derivatives were especially notable, yielding 100% cures at 250 and 200 mg/kg dose levels, respectively. One of the two inactive compounds in this group, 4-pyridyl derivative 17, fails to meet the positional criterion for the side-chain nitrogen atom, and its inactivity is predicted by the hypothesis. The corresponding 2-pyridyl isomer 15 fits the theory and is active. The inactivity of the 4-pyrimidyl derivative 20 is an unexplained exception to the hypothesis, although the presence of aza nitrogen para to the nitrofurylvinyl group (as in 17) may be involved. No other compounds among those evaluated possess this feature. All other positions for second aza moieties (*e.g.*, 18, 21, and 22) appear compatible with activity.

Parallel with the results of Hulbert, *et al.*,[7] we found that the vinyl group is essential to antischistosomal activity. In three cases, replacement of the vinyl bridge of highly active compounds with a carboxamide linkage (compare 9 with 12, 13 with 14, and 18 with 19) gave essentially inactive analogs. This exchange would be expected to only slightly alter the geometrical relationship between the nitrofuran moiety and the other heterocycle (*e.g.*, compare structures 18 and 19). The amide linkage differs chiefly in that it allows rotation about the carbonyl-nitrogen single bond, whereas this is precluded with the corresponding carbon–carbon double bond of the vinyl group.



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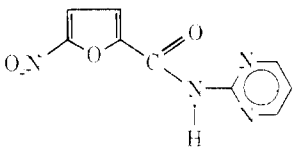

19

Isosteric replacement of the nitrofuran moiety of active compounds with 5-nitro-2-thienyl diminished antischistosomal activity. A small proportion of short-term effects were generally retained in the thiophene analogs (compare 4 with 5, 6 with 7, 15 with 16, and 23 with 24) but this substitution strongly reduced long-term effects on the worms.

A third structural variation that proved unfavorable was the addition of a fused benzene ring to the system. Benzologs 23 and 25, corresponding to the highly active pyrimidine and thiazole derivatives 18 and 8, respectively, were virtually inactive. Benzimidazoles 26 and 27, formally derived from 4 and 6, respectively, retained significant short term effects but were more toxic than the corresponding imidazoles.

As noted previously with most of the nitrofurylacrylamides,[7] activity was evident with several of the compounds reported here (9, 13, 21, 22) but only at toxic dose levels. The dose–response relationship of one of the best compounds found in this study, pyrimidine derivative 18, appears to be very steep. At 200 mg/kg, this compound was 100% curative; at 100 mg/kg, it produced no cures and reduced worm burdens by only 18%. An abrupt drop in effectiveness with decreasing dose is also characteristic for 3.[7,8]

The results reported here affirm the hypothesis that led to the investigation[6] and further define the additional structural features necessary to impart antischistosomal activity to nitroheterocycles. Of the four ring systems now evaluated as carriers of the nitro group (furan, thiazole, thiophene, and phenyl), furan clearly gives superior activity.[6,7] Of the five bridging moieties examined within the context of the hypothesis (vinyl, acetylenic, 1,2-ethylidine, carboxamide, and the amide moiety of niridazole), only the vinyl appears acceptable. The general use of the niridazole-type bridge is uncertain; many analogs derived from 2-amino-5-nitrothiazole have been examined, and activity is found only in a very narrow range.[13] The presence of the specifically placed nitrogen atom in the side chain (provided in this study as an aza moiety of a heterocyclic ring) remains established as a necessary requirement for activity. However, that the nitrogen atom above is insufficient for activity is shown by the lack of activity seen with some compounds such as 20 and 23. The specific need for the nitro group, distinct from other electron-withdrawing groups, was established recently within the antischistosomal nitrofurylacrylamide group.[14] This latter requirement may be associated with biological reduction to an active metabolite. The nitro group of 3 is reduced by

mammalian liver systems,[15-17] and the ease of reduction of a number of nitrofurans has been correlated with their antibacterial activity.[18]

The use of partition coefficients to correlate chemical structures with biological activity has been developed extensively by Leo and Hansch[19] and Hansch.[20] Since an active antischistosomal agent must cross a number of biological membranes to reach the site of action, a correlation should exist between the lipophilicity of drugs (as represented by the partition coefficient) and their activity. A calculation of the $\Sigma\pi$ values of the most active compounds of Table I was done according to Leo and Hansch[19] and Hansch.[20] The values for **8**, **15**, and **18** were 2.1, 1.9, and 1.1, respectively; they are in reasonable agreement with values of 0.8 and 0.6 calculated by Hulbert, *et al.*,[7] for their two most active nitrofurylacrylamides. The calculated $\Sigma\pi$ value for compound **3** is −2.44, however, substantially lower than the values of other active compounds. This lack of correlation could mean that there is more than one optimum $\Sigma\pi$ value in the series, or it could reflect the approximate nature of calculated partition coefficients. A comparison of the contrasting activities of the isomeric pair **18** and **20** clearly shows that factors other than isolipophilicity are involved. Calculated $\Sigma\pi$ values for these compounds are identical.

A major undefined area in the nitrofurylvinyl group of active compounds prepared in this study is the effect of substituents in the nitrogen heterocycle. The intent of the work was to determine the influence of the unsubstituted heterocycle on activity, but a few substituted examples were nevertheless examined. The insertion of an amino group into thiadiazole derivative **9** to give **11** reduced toxicity and enhanced activity. As reported earlier,[8] the desamino analog of **3** was as active as **3** but substantially more toxic. The insertion of acetyl groups into compounds of our series was detrimental in two cases (*e.g.*, **4** *vs.* **6** and **10** *vs.* **11**) but had no influence in the case of **26** and **27**. The *N*-acetyl analog of **3** was as active as the parent.[8] At this time, little can be concluded from these substituent data except that small structural changes may affect activity considerably. The effect of substituents on the more active nitrofurylvinyl heterocycles reported in this study is currently being investigated in an attempt to find derivatives of greater potency and lower toxicity.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had an ir spectrum compatible with its structure. All analytical samples gave combustion values for C, H, and N within 0.4% of theoretical values.

***N*-Acetyl-2-[2-(5-nitro-2-furyl)vinyl]imidazole (6). Method A.** To 14.0 g of 5-nitrofurfural (0.1 mol) was added 8.1 g of 2-methylimidazole (0.1 mol), 80 ml of Ac_2O, and 80 ml of AcOH. The solution was stirred at reflux for 4 hr and cooled to 0°. The resulting precipitate was collected and washed with 100 ml of C_6H_6. The crude product was recrystallized from EtOAc to give 10.6 g (43%) of red crystals, mp 163–164°. (See Table I for additional data on compounds made by this method.)

2-[2-(5-Nitro-2-furyl)vinyl]imidazole (5). Method B. To 2.2 g (0.01 mol) of **6** was added 20 ml of 6 *N* HCl and 10 ml of EtOH. The reaction mixture was stirred at reflux for 1 hr, cooled, and evaporated *in vacuo* to dryness. The residue was recrystallized from CH_3CN to yield 0.778 g (38%) of yellow crystals, mp 223°. (See Table I for additional data on compounds made by this method.)

***N*-(2-Pyrimidinyl)-5-nitro-2-furancarboxamide (19). Method C.** Molecular sieve dried (4 Å) DMF (7.3 g, 0.1 mol) was slowly added at room temperature to a stirred suspension of 5-nitro-2-furoic acid (15.7 g, 0.1 mol) in 75 ml of thionyl chloride. The reaction mixture was heated at reflux for 1.5 hr, cooled, and evaporated *in vacuo* to dryness. The residue was dissolved in 80 ml of C_6H_6 and added dropwise to a suspension of 2-aminopyrimidine in 80 ml of C_6H_6. The mixture was heated at reflux for 1 hr, cooled, and evaporated *in vacuo* to dryness. The residue was treated with warm H_2O and filtered. The product was recrystallized twice from 50% aqueous EtOH with charcoal treatment to give 2.8 g (69%) of solid, mp 210–211°. (See Table I for additional data on compounds.)

Acknowledgment. This work was generously supported by Grants AI-08022, GM-16492, and AI-07962 from the National Institutes of Health.

References

(1) L. M. Werbel in "Topics in Medicinal Chemistry," Vol. 3, J. A. Rabinowitz and M. Meyerson, Ed., Wiley-Interscience, New York, N. Y., 1970.
(2) P. Jordan, Symposium on Schistosomiasis, *Bull. N. Y. Acad. Med.*, **44**, 245 (1968).
(3) H. M. Gilles, *Practitioner*, **200**, 451 (1968).
(4) C. Hsueh-chang, H. Ming, C. Hsiao-lan, and W. Man-hua, *Chin. Med. J.*, **84**, 591 (1965).
(5) C. Hsueh-chang, H. Ming, and C. Hsiao-lan, *ibid.*, **82**, 250 (1963).
(6) C. H. Robinson, E. Bueding, and J. Fisher, *Mol. Pharmacol.*, **6**, 604 (1970).
(7) P. B. Hulbert, E. Bueding, and C. H. Robinson, *J. Med. Chem.*, **16**, 72 (1973).
(8) E. Bueding, C. Naquira, S. Bouwman, and G. Rose, *J. Pharmacol. Exp. Ther.*, **178**, 402 (1971).
(9) G. D. Erickson, V. G. Bourgeous, E. H. Sadun, and E. Bueding, *ibid.*, **178**, 411 (1971).
(10) E. Bueding and J. Fisher, *Mol. Pharmacol.*, **6**, 532 (1970).
(11) J. Bourgeois and E. Bueding, *J. Pharmacol. Exp. Ther.*, **176**, 455 (1971).
(12) R. W. Lennox and E. Bueding, *Amer. J. Trop. Med. Hyg.*, **21**, 302 (1972).
(13) L. M. Werbel and J. R. Battaglia, *J. Med. Chem.*, **14**, 10 (1971).
(14) C. H. Robinson, S. Spengel, and E. Bueding, *ibid.*, **16**, 79 (1973).
(15) D. R. Feller, M. Marita, and J. G. Gillette, *Biochem. Pharmacol.*, **20**, 203 (1971).
(16) M. Marita, D. R. Feller, and J. G. Gillette, *ibid.*, **20**, 217 (1971).
(17) D. R. Feller, M. Marita, and J. G. Gillette, *Proc. Soc. Exp. Biol. Med.*, **137**, 433 (1971).
(18) H. Kala and D. Ausborn, *Pharmazie*, **26**, 121 (1971).
(19) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(20) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).