the aromatic ring and the iodoacetamide moiety in these reagents did not affect the first-order rate constants for inactivation of COMT. Also, the simple observation that modification of this amino acid moiety results in loss of enzymatic activity would imply a crucial role for this functional group in catalysis or binding. Our earlier observation[4] that inclusion of catechol substrate in the preincubation mixture protects the enzyme from inactivation by the *N*-iodoacetyl derivative 4 provides further evidence that an active site amino acid is being modified and that these compounds are specific active-site-directed alkylating reagents. Using the information obtained in this study, we are now carrying out incorporation studies and attempting to isolate and characterize the modified amino acid residues.

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Antiparasitic Nitroimidazoles. 8. Derivatives of 2-(4-Formylstyryl)-5-nitro-1-vinylimidazole

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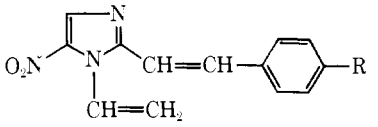
A series of 33 thioacetals and hydrazones of 2-(4-formylstyryl)-5-nitro-1-vinylimidazole was prepared and examined for antitrypanosomal properties. The thioacetals were inactive as antitrypanosomal agents but three hydrazones derived from *N*-aminoguanidine, pyridylacetohydrazide chloride (Girard reagent P), and dimethylaminoacetohydrazide (Girard reagent D) displayed good activity against *Trypanosoma rhodesiense*.

In part 3[1] we described the antitrypanosomal properties of 2-(4-carboxystyryl)-5-nitro-1-vinylimidazole (1a) and a number of related compounds including the aldehyde **1b**.



1a, R = COOH
b, R = CHO
c, R = -CH(O)O

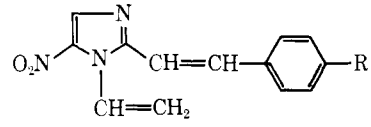
The latter compound was equiactive with 1a against *Trypanosoma rhodesiense* infections in mice when dosed ip but was considerably less active when dosed orally.[1] However, it was shown[2] that **1b** is rapidly metabolized in the mouse to 1a and excreted as its glucuronide.[3] We considered that if **1b** could be suitably derivatized we might accomplish two things: (a) increase the intrinsic activity of the compounds against trypanosomes; (b) prevent rapid metabolism and excretion of the compound.

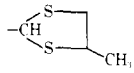
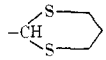
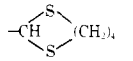
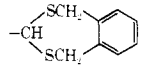
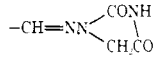
Chemistry. It was already known[1] that 1c had reasonable activity against a number of *Trypanosoma* species so we prepared by standard methods (see Experimental Section) a number of thioacetals (2–7) derived from **1b** which we considered would be metabolically and chemically more stable than 1c. The hydrazones 8–19 were prepared by analogy with the many hydrazones derived from 5-nitrofurfuraldehyde and related compounds.[4,5] In particular, compounds **10**, **11**, and **12** were prepared as analogs of nitrofurazone, nitrofurantoin, and guanofuracin, respectively. Nitrofurazone has been shown to be effective against *Trypanosoma gambiense* infections in guinea pigs[6] and to inhibit a *Trypanosoma cruzi* infection in mice.[7] Compounds 13–16 can be considered as analogs of nifurtimox,[8] a promising compound for the treatment of acute and chronic Chagas disease (Table I).

The quaternary acylhydrazones **20** and **24** were prepared in an attempt to impart some water solubility to the compounds and also as analogs of 5-nitro-2-furfuraldehydetrimethylammonium acetylhydrazone chloride which has been shown to inhibit inections of *T. cruzi* in mice.[7]

Biological Results. All the compounds were tested against infections of *T. rhodesiense* in mice using the procedures described by Hawking.[9] If the compounds showed activity in this test they were tested against *T. cruzi*, *T. gambiense*, and *Trypanosoma congolense* by a similar procedure. Only compounds active against one or more of the above organisms are listed in Table II. None of the thioacetals 2–7 showed activity comparable to 1c and this series was abandoned. The simple hydrazones 8 and 9 were both inactive as was **10**, the analog of nitrofurazone. The nitrofurantoin analog **11** exhibited marginal activity against *T. rhodesiense*, *T. gambiense*, and *T. congolense* but was inactive against *T. cruzi*. The guanofuracin ana-

Table I



Compd	R	Yield, %	Crystn solvent	Mp, °C	Formula[a]
2		74	EtOH	143–144	$C_{16}H_{15}N_3O_3S$
3		78	EtOH	146–147	$C_{16}H_{15}N_3O_2S_2$
4		62	EtOH	114–115	$C_{17}H_{17}N_3O_2S_2$
5		73	$CHCl_3$–pet. ether	221–222	$C_{17}H_{17}N_3O_2S_2 \cdot 0.5H_2O$
6		58	$CHCl_3$–pet. ether	169–170	$C_{18}H_{19}N_3O_2S_2$
7		64	$CHCl_3$–MeOH	255–256	$C_{24}H_{23}N_3O_2S_2$
8	$-CH=NNHC_6H_5$	95	EtOH	216–217	$C_{20}H_{17}N_5O_2$
9	$-CH=NNH\text{-}2,4\text{-}(NO_2)_2C_6H_3$	86	AcOH	298–299 dec	$C_{20}H_{15}N_7O_6$
10	$-CH=NNHCONH_2$	64	Dioxane	261–262 dec	$C_{15}H_{14}N_6O_3$
11		79	DMF	283–284 dec	$C_{17}H_{14}N_6O_4 \cdot 0.5H_2O$
12	$-CH=NNHC(=NH)NH_2$	81	$DMF \cdot H_2O$	248–250 dec	$C_{15}H_{15}N_7O_2$
13	$-CH=N\text{-}c\text{-}N(CH_2CH_2)_2O$	90	$CHCl_3$	196–197	$C_{18}H_{19}N_5O_3$
14	$-CH=N\text{-}c\text{-}N(CH_2CH_2)_2S$	86	$CHCl_3$–pet. ether	201–202	$C_{18}H_{19}N_5O_2S$
15	$-CH=N\text{-}c\text{-}N(CH_2CH_2)_2SO_2$	84	Me_2CO	268–269	$C_{18}H_{19}N_5O_4S$
16	$-CH=N\text{-}c\text{-}N(CH_2)_5$	76	EtOAc	215–216	$C_{19}H_{21}N_5O_2$
17	$-CH=N\text{-}c\text{-}(CH_2CH_2)_2NCH_2CH_2OH$	82	EtOAc	191–192	$C_{20}H_{24}N_6O_3$
18	$-CH=NN(CH_2CH_2OH)_2$	90	$CHCl_3$–pet. ether	187–188	$C_{18}H_{19}N_5O_4$
19	$-CH=NNHCH_2CO_2Et$	72	$(CH_2OMe)_2$	175–176	$C_{18}H_{19}N_5O_4$
20	$-CH=NNHCOCH_2\text{-}c\text{-}\overset{+}{N}C_5H_5\ Cl^-$	53	$MeCO\text{–}H_2O$	274–276	$C_{21}H_{19}ClN_6O_3 \cdot 2H_2O$
21	$-CH=NNHCOCH_2\text{-}2\text{-}Py$	55	$DMF\text{–}H_2O$	192–193	$C_{21}N_{18}N_6O_3$
22	$-CH=NNHCOCH_2\text{-}3\text{-}Py$	76	$DMF\text{–}H_2O$	248–250	$C_{21}H_{18}N_6O_3$
23	$-CH=NNHCOCH_2\text{-}4\text{-}Py$	62	$DMF\text{–}H_2O$	225–266 dec	$C_{21}H_{18}N_6O_3$
24	$-CH=NNHCOCH_2\overset{+}{N}(CH_3)_3\ Cl^-$	66	$Me_2CO\text{–}H_2O$	235–237	$C_{19}H_{13}ClN_6O_3 \cdot 2H_2O$
25	$-CH=NNHCOCH_2N(CH_3)_2$	66	$CHCl_3$–pet. ether	190–191	$C_{18}H_{20}N_6O_3$
26	$-CH=NNHCOCH_2N(C_2H_5)_2$	52	EtOAc	155–157	$C_{20}H_{24}N_6O_3$
27	$-CH=NNHCOCH_2NHCH_3$	49	EtOAc	153–154 dec	$C_{17}H_{18}N_6O_3$
28	$-CH=NNHCOCH_2NHC_2H_5$	58	EtOAc	183–184	$C_{18}H_{20}N_6O_3$
29	$-CH=NNHCOCH_2\text{-}c\text{-}NC_4H_8$	70	$CHCl_3$–pet. ether	214–215	$C_{20}H_{22}N_6O_3$
30	$-CH=NNHCOCH_2\text{-}c\text{-}NC_5H_{10}$	70	$CHCl_3$–pet. ether	214–215	$C_{20}H_{22}N_6O_3$
31	$-CH=NNHCOCH_2\text{-}c\text{-}N(CH_2CH_2)_2O$	75	$CHCl_3$–pet. ether	219–220	$C_{20}H_{22}N_6O_4$
32	$-CH=NNHCOCH_2\text{-}c\text{-}N(CH_2CH_2)_2SO_2$	68	DMF–EtOH	253–254	$C_{20}H_{22}N_6O_5S$
33	$-CH=NNHCOCH_3$	52	$DMF\text{–}H_2O$	270–272 dec	$C_{16}H_{15}N_5O_3$
34	$-CH=NNHCOPh$	71	DMF	249–250	$C_{21}H_{17}N_5O_3$

[a] All compounds were analyzed for C, H, and N.

log 12 showed good activity ip against *T. rhodesiense* and *T. cruzi* but was less effective po.

The nifurtimox analogs 13–16 proved active ip but were generally less active than the parent compound **1b**. In an attempt to improve water solubility the hydroxyethyl compounds 17 and 18 were prepared. Compound 17 proved reasonably active but 18, the open-chain analog of 13, was inactive as was the ester **19**. We next considered the preparation of acylhydrazones derived from the Girard "T" and "P" reagents; such derivatives would serve the dual purpose of being water soluble and be analogs of an active compound (*vide supra*). Compound **20** was active against *T. rhodesiense*, *T. gambiense*, and *T. congolense* but we were unable to determine the dose which would give a parasitological cure against *T. cruzi* due to toxicity problems. However, the activity of **20** against *T. rhodesiense*, *T. gambiense*, and *T. congolense* warrents special comment as it approaches that of the standard drugs (see Table II). However, **20** is somewhat toxic (LD_{50} ~50 mg/kg ip in mice) and this limits its usefulness. As we considered the toxicity of **20** to be due to the presence of the quaternary function, the pyridylacethydrazones **21**–**23** were prepared as nonquaternary analogs but all were inactive. The trimethylammonium analog of **24** was less active than **20** but the Girard "D" derivative **25** proved to have twice the activity of **1a** and **1b** against *T. rhodesiense* but was inac-

Table II. Minimum Dose Level in mg/kg Shown to Be 100% Effective against Trypanosomal Infections in Mice

Compd	*T. rhodesiense*		*T. cruzi*[b]		*T. gambiense*,[a]	*T. congolense*,
	ip	po	ip	po	ip	ip
1a	25	25	100	200	12.5	25
1b	25	200	500	500	25	50
1c	50	100	200	500	25	100
2	50	200	200	ND	ND	100
3	50	ND	100	ND[c]	ND	ND
4	25	Inact[d] at 25	80	ND	ND	ND
6	50	Inact[d] at 50	Inact at 50	ND	ND	ND
11	200	ND	Inact at 200	ND	100	200
12	12.5	100	50	Inact at 100	ND	25
13	100	Inact at 500	100	Inact at 100	25	200
14	100	Inact at 100	100	Inact at 100	25	100
15	200	Inact at 200	200	Inact at 200	200	200
16	200	ND	Inact at 200		ND	ND
17	50	100	Inact at 50		100	100
18	200	Inact at 200	Inact at 200		ND	ND
20	3	10	Inact at 10	ND	5	10
24	10	10	Inact at 10	ND	10	10
25	12.5	12.5	Inact at 50		ND	50
26	25	25	Inact at 25		ND	ND
29	25	25	ND	ND	ND	ND
31	25	25	Inact at 25		ND	ND
Suramin	1	Inact at 1	Inact at 5	ND	5	Inact at 5
Pentamidine	1.25	Inact at 1.25	Inact at 5	ND	5	5
Diminazene	1	Inact at 1	Inact at 10	ND	5	10
Melarsoprol	0.75	0.5	Inact at 2	ND	0.75	Inact at 2

[a] Mice were dosed for four consecutive days, commencing on the day of infection. 100% efficacy is equivalent to 30-day post-infection survival with negative parasitemia. [b] Mice were dosed for five consecutive days, commencing on the day of infection. 100% efficacy is equivalent to 60-day post-infection survival with negative parasitemia. [c] ND = not done. [d] Compounds were defined as inactive if they failed to prolong the mean survival time (MST) of infected mice to the time scale defined in footnotes *a* and *b*. MST for control animals were as follows: *T. rhodesiense* and *T. gambiense*, 4 days; *T. cruzi*, 13–14 days; *T. congolense*, 7–10 days.

tive against *T. cruzi*. A detailed description of the activity of **25** is given in Table III. A number of congeners of **25** were prepared in order to define the requirements for activity. The *N*-diethyl analog **26** was somewhat less active than **25** while the secondary amines **27** and **28** were both inactive. Introduction of a pyrrolidine function to give **29** reduced activity while the presence of a piperidine **30** function resulted in loss of activity. The morpholino compound **31** had similar activity to **29** but the thiomorpholine dioxide **32** was completely inactive. Replacement of the tertiary amine function in **25** by a methyl **33** or phenyl **34** groups gave inactive compounds.

It would appear, therefore, that maximum antitrypanosomal activity for these acylhydrazones resides in compound **25** and any change in the structure of the hydrazone function is detrimental to activity.

In conclusion, we were able to prepare a number of derivatives of **1b** (*e.g.*, **12**, **20**, and **25**) which are more active against *T. rhodesiense* than the parent compound or our previous best compound **1a**. However, in general, the compounds had poor activity against *T. cruzi* and none were quite as active against *T. rhodesiense* as the standard drugs suramin, pentamidine, diminazene, or melarsoprol.

Table III. Per Cent Activity[a] of Compound 25 against African Trypanosomes

T. rhodesiense			*T. gambiense*			*T. congolense*		
Dose, mg/kg	Route	% act.	Dose, mg/kg	Route	% act.	Dose, mg/kg	Route	% act.
12.5 × 4	ip	100	10 × 4	po	100	50 × 4	ip	79
10 × 4	po	100	25 × 1	po	100	25 × 4	sc	100
50 × 1	po	89						
25 × 1	po	97						
2 × 4	sc	100						
5 × 1	sc	80						

[a] % activity calculated as on Table II.

Experimental Section

Melting points were taken on a Gallenkamp apparatus (Registered Design No. 889339) using capillaries and are uncorrected. All compounds were characterized by ir, uv, nmr, and elemental analyses (C, H, N) which were within ±0.4% of the theoretical values.

Acethydrazide, benzhydrazide, and Girard reagents "T" and "P" were purchased from BDH; Girard reagent "D" was obtained from Eastman-Kodak. All the other required hydrazides were prepared by literature methods.[10]

The hydrazines used for the preparation of compounds 13–18 were prepared by literature methods[11] and kindly supplied by Mr. J. P. Verge.

General Method for Thioacetals. The aldehyde **1b** (0.05 mol) was refluxed with the appropriate thiol (0.05 mol) in benzene and in the presence of *p*-toluenesulfonic acid (100 mg) for 2 hr, the water from the reaction being removed *via* a Dean-Stark separator. After cooling, the benzene solution was washed with $NaHCO_3$ solution, followed by H_2O, and then evaporated *in vacuo* to give a bright yellow solid which was crystallized from an appropriate solvent, *e.g.*, ethanol.

General Method for Hydrazones. The aldehyde **1b** (0.025 mol) and the hydrazine or hydrazide (0.025 mol) were refluxed in a solvent such as EtOH or $CHCl_3$, with or without the addition of a few drops of glacial acetic acid, for 2–4 hr. On cooling the resultant crystalline solid was filtered off and recrystallized from an appropriate solvent.

2-(4-*N*,*N*-Dimethylglycinamidoiminomethinestyryl)-5-nitro-1-vinylimidazole (25). Aldehyde **1b** (6.7 g, 0.025 mol), *N*,*N*-dimethylglycine hydrazide hydrochloride (3.8 g, 0.025 mol), and sodium acetate hydrate (4 g) were refluxed in EtOH (300 ml) for 2 hr. On cooling a small amount of inorganic material separated and was filtered off. The filtrate was evaporated *in vacuo* to give an oil which on treatment with $CHCl_3$ gave further inorganic solid which was filtered off. The bright yellow filtrate was carefully diluted with petroleum ether (bp 40–60°) and allowed to crystallize: yield 6.1 g (66%); mp 190–191°.

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Synthesis and Biological Evaluation of Xanthine Oxidase Inhibitors. Pyrazolo[3,4-*d*]pyrimidines and Pyrazolo[3,4-*b*]pyridines†

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1-, 3-, and 5-substituted pyrazolo[3,4-*d*]pyrimidines and pyrazolo[3,4-*b*]pyridines related to allopurinol were synthesized and evaluated as xanthine oxidase inhibitors. Among these compounds, 4-hydroxypyrazolo[3,4-*b*]pyridine-5-carboxylic acids **12** were found to possess potency in the same order of allopurinol. The influence of the substitutions on the enzyme inhibitory effect and the bulk tolerance of the enzyme–inhibitor complex are discussed.

4-Hydroxypyrazolo[3,4-*d*]pyrimidine (allopurinol, **1a**) has been shown to be an effective inhibitor of xanthine oxidase and thus a clinically useful agent in gout treatment; it is also an effective adjuvant in antitumor chemotherapy.[1] The objective of the work now reported is to develop more effective enzyme inhibitors using the parent skeleton (pyrazolo[3,4-*d*]pyrimidine) of allopurinol as the basis species, both by exploring the effects of substituent insertion on the activity of allopurinol and also by exploring various deaza species related to this molecule. Previ-

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‡ Postdoctorate Fellow, 1972–1974.