xanthone-2-carboxylic acids (23) were converted into alkoxyxanthone carboxylic acids (25a–e) by ether cleavage with HBr in acetic acid followed by alkylation of the resulting phenol.

Experimental Section

General Methods. Phenols were coupled with aromatic halides using Cu_2O[8] in dimethylacetamide (Schemes II, III, and V) and copper powder[10] with anhydrous potassium carbonate in dimethylformamide (Scheme IV). Dicarboxylic acids were cyclized to xanthonecarboxylic acids by polyphosphoric acid[9] in tetramethylene sulfone or with concd H_2SO_4. Methyl diphenyl ethers were oxidized to the corresponding diphenylcarboxylic acids by $KMnO_4$ in *tert*-BuOH–H_2O. Methanolysis of 6-chloroxanthone-2-carboxylic acid (18a) was performed as described by Goldberg and Wragg,[9] except that HMPA was used as solvent.

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References

(1) C. P. Huttrer and E. Dale, *Chem. Rev.*, 48, 543 (1951).
(2) J. S. G. Cox, *Nature (London)*, 216, 1328 (1967).
(3) H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, 15, 583 (1972).
(4) I. Mota, *Immunology*, 7, 681 (1964).
(5) G. Goose and A. M. J. N. Blair, *ibid.*, 16, 749 (1969).
(6) Allen and Hanburys Limited, Belgian Patent 759,292 (1971).
(7) J. S. H. Davies, F. Scheinmann, and H. Suschitzky, *J. Chem. Soc.*, 2140 (1956).
(8) R. J. R. Bacon and O. J. Stewart, *ibid.*, 4953 (1965).
(9) A. A. Goldberg and A. H. Wragg, *ibid.*, 4227 (1958).
(10) H. Meerwein in "Methoden Der Organischen Chemie," Vol. 6, Part 3, Verlag, Stuttgart.
(11) A. M. El-Abbady, S. Ayoub, and F. G. Baddar, *J. Chem. Soc.*, 2556 (1960).
(12) T. Sengoku, *Yakugaku Zasshi*, 53, 962 (1933).

Antiparasitic Nitroimidazoles. 1. Some 2-Styryl-5-nitroimidazoles

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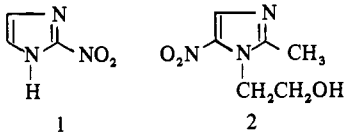
A series of 1-substituted-2-styryl-5-nitroimidazoles was prepared by condensing 1-substituted-2-methyl-5-nitroimidazoles with aryl aldehydes in the presence of sodium methoxide. The aryl aldehydes were substituted with aryl, alkyl, alkoxyl, and chloro groups while the 1 substituent on the imidazole nucleus varied from alkyl to hydroxyalkyl to alkylene. The alkylene substituent was introduced by base elimination of the tosylates of the hydroxyalkyl compounds. A number of arylethynyleneimidazoles were also prepared by bromination of the styryl compound followed by didehydrobromination with DBN. All the compounds were tested against *Trichomonas vaginalis* and *Entamoeba histolytica in vitro* and *in vivo* and against various *Trypanosoma* species *in vivo*. Structure–activity relationships are discussed and comparisons of biological activity made with established drugs.

Since the discovery of the antibacterial and antiprotozoal activity of the antibiotic azomycin, **1**,[1-3] and the subsequent introduction of metronidazole,[4] **2**, as a clinically effective trichomonicide, many papers[5] have appeared on the biological properties of nitroimidazoles.

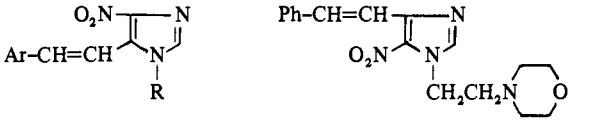


1 2

Although several papers and patents[6-8] have described the preparation of styrylimidazoles and their antitrichomonal and antiamoebic properties *in vitro* none of the compounds so far discussed appear to have *in vivo* activity against these protozoa. In an early paper, Ellis, *et al.*,[6] described the preparation of **3a** and its *in vitro* activity against *Trichomonas vaginalis* but no *in vivo* activity was reported. Similarly, during the course of our work, Giraldi and his coworkers[7] described a series of styrylimidazoles, exemplified by **3b** and **4**, which, although very active against *T. vaginalis* and *Entamoeba histolytica in vitro*, were devoid of *in vivo* activity.

In this paper we describe a series of 2-styryl-5-nitroimidazoles which exhibit a wide range of antiprotozoal activity both *in vitro* and *in vivo* against *T. vaginalis*, *E. histolytica*, and various *Trypanosoma* species.

Chemistry. The 2-styryl-5-nitroimidazoles were all prepared by the general route shown in Scheme I followed by modification of the N substituent in appropriate cases.



3 4

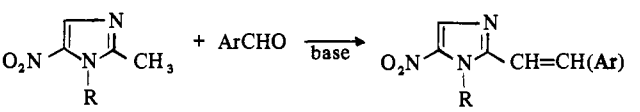
a, Ar = 3,4-$(Cl)_2$Ph; R = Me

b, Ar = Ph; R = CH_2CH_2NO

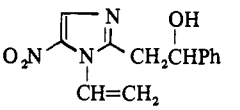
The *N*-vinyl series (Table IV) could be prepared by condensing the aromatic aldehyde with **5** (R = $CH{=}CH_2$) or by base elimination of the tosylate derived from **6** (R = CH_2CH_2OH).

In general, it was better to prepare the *N*-vinyl series by the latter method because compounds of type **6** (R = vinyl)

Scheme I



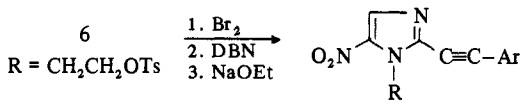
5 6



7

R = alkyl, hydroxylalkyl, or vinyl

Scheme II



8
a, R = CH_2CH_2OTs
b, R = $CH{=}CH_2$

are less stable than the hydroxyalkyl derivatives toward strong bases.[9] Condensation of benzaldehyde and **5** (R = $CH{=}CH_2$) at room temperature resulted in the isolation of the intermediate alcohol **7** which was easily dehydrated to the required compound **54** (Table IV). In other cases the styryl compound was obtained directly. The methods described in the Experimental Section for the preparation of **5** (R = Et) and **5** (R = $CH{=}CH_2$) are superior to literature methods.[10] The acetylenes were prepared as in Scheme II.

The use of the mild base 1,5-diazobicyclo[3.4.0]non-5-ene (DBN)[11] as the dehydrohalogenating agent is noteworthy as in general it is necessary to use a strong base to generate acetylenes from dihaloalkanes.[12] The presence of the nitroimidazole and phenyl rings presumably activates the hydrogen atoms toward elimination reactions. Since all the styrylimidazoles we prepared had the trans configuration, we tried to prepare a compound with a *cis*-styryl linkage. However, attempts to convert the trans compound **31** (Table II) into the cis isomer photochemically were of no avail. Reduction of the acetylene **8a** (Ar = 4-MePh) with disiamylborane,[13] a reagent which normally converts acetylenes into cis olefins, gave only a small yield of the trans olefin **31** (Table II), an observation which we cannot explain since Dreiding models imply little steric overcrowding in the cis olefin.

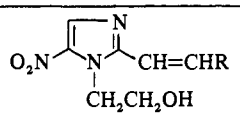
Biological Results. Trichomoniasis. It will be seen from Tables I-V that nearly all compounds inhibited the growth of *T. vaginalis in vitro* at similar levels to metronidazole. No particular substitution pattern seems to favor high *in vitro* activity although in general the tosylates (Table II) and the acetylenes (Table V) appear to be the least active. Introduction of a long alkyl residue into the benzene ring, *e.g.*, compounds **21**, **39**, and **69**, abolished activity. Compounds **52** and **53** were prepared as analogs of compounds described by Giraldi[7] and in common with many other compounds in Tables I-V were inactive against *T. vaginalis* in mice when tested according to the method described by Honigberg.[14] None of the compounds were as active as an aqueous suspension of metronidazole against *T. vaginalis* in mice at 20 mg/kg X 5 po (Table VI) and all suffered the disadvantage of having to be dosed in PEG400 to obtain maximum activity. Compounds which resulted in less than a 50% reduction in lesion score, compared with the score in untreated infected mice, were considered inactive. In this test metronidazole gave a 100% reduction at 20 mg/kg X 5 po.

It would appear from the *in vivo* (Table VI) results that only those compounds containing a *p*-alkyl substituent in the benzene ring and having an *N*-vinyl substituent in the nitroimidazole residue show significant activity. The partial exception is compound **50** which contains a *p*-isopropyl group in the benzene ring, but an *N*-Me substituent in the nitroimidazole. Compounds **81**, **82**, and **83** all meet the above criteria, but the benzene ring and the nitroimidazole are joined by an acetylenic instead of an olefinic linkage. This leads to the supposition that for activity the nature of the linkage between the two rings was not of supreme importance provided that they were conjugated. A recent report describes a number of 2-phenyl-5-nitroimidazoles having exceptional antitrichomonal and amoebicidal activity.[15]

Amoebiasis. The assessment of antiamoebic activity was based on methods described by Jones[16] for intestinal amoebiasis in rats and Reinertson and Thompson[17] for hepatic amoebiasis in hamsters.

The compounds were much less active against *E. histolytica* in mice and hamsters than metronidazole (Table VI), but the structure–activity relationship was similar to that shown against *T. vaginalis*. In this instance the acetylenes

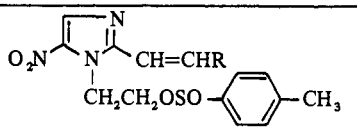
Table I



Compd	R	Yield, %[c]	Mp, °C	Formula[h]	MIC, μg/ml[i] *T. vaginalis*	MIC, μg/ml[i] *E. histolytica*
9	Ph[a]	32[d]	156–157	$C_{13}H_{13}N_3O_3$	8.0	8.0
10	2-ClPh[a]	41	188–189	$C_{13}H_{12}ClN_3O_3$	1.0	8.0
11	4-ClPh[a]	37	194–195	$C_{13}H_{12}ClN_3O_3$	0.5	8.0
12	2-CH_3Ph[a]	36	159–160	$C_{14}H_{15}N_3O_3$	0.25	16.0
13	4-CH_3Ph[a,b]	21 (77)	145–146	$C_{14}H_{15}N_3O_3$	2.0	32.0
14	3,4-$(CH_3)_2$Ph[b]	50	159–160	$C_{15}H_{17}N_3O_3$	0.5	32.0
15	2,4,6-$(CH_3)_3$Ph[b]	25	148–149	$C_{16}H_{19}N_3O_3$	8.0	16.0
16	4-C_2H_5Ph[b]	40	187–188	$C_{15}H_{17}N_3O_3$	0.5	16.0
17	4-*i*-C_3H_7Ph[a]	21	171–172	$C_{16}H_{19}N_3O_3$	0.5	16.0
18	4-*tert*-C_4H_9Ph[a]	32[e]	152–153	$C_{17}H_{21}N_3O_3$	1.0	32.0
19	4-*sec*-C_4H_9Ph[b]	42[f]	102–103	$C_{17}H_{21}N_3O_3$	0.5	32.0
20	4-*cyclo*-C_6H_{11}Ph[b]	27[g]	53–55	$C_{19}H_{23}N_3O_3$	2.0	64.0
21	4-*n*-C_8H_{17}Ph[b]	24	92–93	$C_{21}H_{29}N_3O_3$	>1000	>1000
22	4-PhPh[b]	79	201–202	$C_{19}H_{17}N_3O_3$	2.0	16.0
23	1-Naphthyl	40	192–193	$C_{17}H_{15}N_3O_3$	1.0	16.0
24	2-Naphthyl	73	196–197	$C_{17}H_{15}N_3O_3$	1.0	32.0
25	2-MeOPh[b]	82	199–200	$C_{14}H_{15}N_3O_4$	0.5	8.0
26	3-MeOPh[b]	72	134–135	$C_{14}H_{15}N_3O_4$	0.25	8.0
27	4-MeOPh[a]	19	152–153	$C_{14}H_{15}N_3O_4$	4.0	16.0
28	3,4,5-$(MeO)_3$Ph[b]	38	152–153	$C_{16}H_{19}N_3O_6$	0.5	16.0
2	Metronidazole				0.5	32.0

[a,b]Prepd by method A or B. [c]All compds were recrystd from EtOH unless otherwise noted. Recrystn solvents were: [d]EtOAc; [e]EtOH–H_2O; [f]EtOH–petr ether; [g]EtOAc–petr ether. [h]All compds were analyzed for C, H, N. [i]Detd by serial diln *in vitro*.

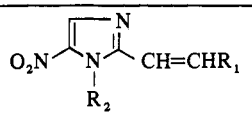
Table II



Compd	R	Yield, %[a]	Mp, °C	Formula[d]	MIC, μg/ml[e] *T. vaginalis*	MIC, μg/ml[e] *E. histolytica*
29	2-ClPh	89[b]	210–212 dec	$C_{20}H_{18}ClN_3$	8.0	128
30	2-CH_3Ph	85	177–178	$C_{21}H_{21}N_3O_5S$	4.0	128
31	4-CH_3Ph	61	155–156	$C_{21}H_{21}N_3O_5S$	2.0	128
32	3,4-$(CH_3)_2$Ph	13	172–173	$C_{22}H_{23}N_3O_5S$	8.0	128
33	2,4,6-$(CH_3)_3$Ph	66	154–155	$C_{23}H_{25}N_3O_5S$	16.0	32
34	4-C_2H_5Ph	87	134.5–135.5	$C_{22}H_{23}N_3O_5S$	1.0	32
35	4-*i*-C_3H_7Ph	71	148–149	$C_{23}H_{25}N_3O_5S$	4.0	64
36	4-*tert*-C_4H_9Ph	57	146–147	$C_{24}H_{27}N_3O_5S$	4.0	>1000
37	4-*sec*-C_4H_9Ph	86[c]	119–120	$C_{24}H_{27}N_3O_5S$	4.0	64
38	4-*cyclo*-C_6H_{11}Ph	83	129–130	$C_{26}H_{26}N_3O_6S$	4.0	100–1000
39	4-*n*-C_8H_{17}Ph	80	130–131	$C_{28}H_{35}N_3O_5S$	>1000	>1000
40	4-PhPh	52	169–170	$C_{26}H_{23}N_3O_5S$	2.0	8.0
41	1-Naphthyl	37	169–170	$C_{24}H_{21}N_3O_5S$	1.0	32.0
42	2-Naphthyl	56	186–187	$C_{24}H_{21}N_3O_5S$	2.0	100–1000
43	2-MeOPh	75	169–170	$C_{21}H_{21}N_3O_6S$	2.0	100–1000
44	3-MeOPh	81	185–188	$C_{21}H_{21}N_3O_6S$	100–1000	>1000
45	3,4,5-$(MeO)_3$Ph	74	159–160	$C_{23}H_{25}N_3O_8S$	2.0	32

[a] All compds were crystd from EtOH except where noted. [b] Recrystd EtOAc. [c] Recrystd EtOAc–petr ether. [d] All compds were analyzed for C, H, N. [e] Determined by serial diln.

Table III



Compd	R_1	R_2	Yield, %[a,l]	Mp, °C	Formula[g]	MIC, μg/ml[h] *T. vaginalis*	MIC, μg/ml[h] *E. histolytica*
46	Ph	Me	51[b]	198–199	$C_{12}H_{11}N_3O_2$	8.0	8.0
47	Ph	Et	50[c]	146–148	$C_{13}H_{13}N_3O_2$	1.0	8.0
48	4-MePh	Me	57[d]	217–218	$C_{13}H_{13}N_3O_2$	4.0	100–1000
49	4-MePh	Et	35[c]	127–129	$C_{14}H_{15}N_3O_2$	1.0	16.0
50	4-*i*-C_3H_7Ph	Me	12[b]	142–143	$C_{15}H_{17}N_3O_2$	1.0	32
51	4-*i*-C_3H_7Ph	Et	25	94–95[e]	$C_{16}H_{19}N_3O_2$	1.0	32
52	4-MePh	CH_2CH_2N⟨O⟩	9	125–126	$C_{18}H_{22}N_4O_3$	0.2	Inactive
53	4-MePh	CH_2CH_2Cl	77[f]	159–160	$C_{14}H_{14}ClN_3O_2$	0.5	

[a] Recrystn solvents. [b] EtOH. [c] EtOAc–petr ether. [d] EtOAc. [e] Petr ether. [f] $CHCl_3$–DMF. [g] All compds were analyzed for C, H, N. [h] Determined by serial diln. [i] Prepd by method F.

81, **82**, and **83** were inactive against the intestinal form of amoebiasis in the rat.

Trypanosomiasis. All compounds were tested against infections of *Trypanosoma rhodiesiense*, *Tryp. cruzi*, *Tryp. gambiense*, and *Tryp. congolense* in mice using the procedures described by Hawking.[18] Only compounds listed in Tables III, IV, and V showed activity against these organisms *in vivo*. Compound **54** showed marginal activity against *Tryp. rhodiesiense*, *Tryp. gambiense*, and *Tryp. congolense* (Table VII) and was, in fact, the lead compound.

In an attempt to improve this activity and to delineate the structural requirements for antitrypanosomal activity, compound **54** was modified in several ways. The molecule was divided into three substructures (see diagram) which could be varied independently or in unison.

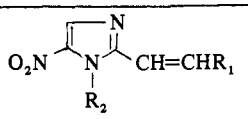
Introduction of chlorine atoms into the benzene ring gave compounds **74–76** which were devoid of antitrypanosomal activity as were the methoxy-substituted compounds, **77–80** (Table IV).

Substitution of the benzene ring with a 4-Me group to give **55** resulted in an increase in antitrypanosomal activity *vis-a-vis* **54**, and it is of interest that this compound showed activity against *Tryp. cruzi*, the causative organism of Chagas' disease. The 2-Me isomer (**59**) of **55** was inactive against all the trypanosomes while substitution of **55** with more methyl groups, to give **57** and **58**, resulted in the diminution and abolition of antitrypanosomal activity, respectively. Extension or branching the 4-alkyl residue gave a number of compounds, notably **65** and **68**, with a similar spectrum and level of activity to **55**. However, when the chain was extended to C_8 as in **69**, activity was greatly reduced while the *tert*-Bu compound **62** was completely inactive. Replacement of the *N*-vinyl group by an *N*-propenyl function gave compounds with similar activity to the parent compounds.

The *N*-alkyl analogs **48–51** were all less active than the corresponding *N*-vinyl compounds, while **46** and **47**, analogs of the lead compound, were inactive. Replacement of the olefinic linkage (B) by an acetylenic linkage to give compounds **81–83** resulted in a marked reduction in activity against trypanosomes.

From these results it was concluded that for maximum antitrypanosomal activity in this series of compounds, the

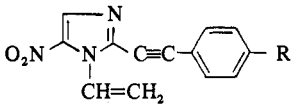
Table IV



Compd	R_1	R_2	Yield, %[a]	Mp, °C	Formula[c]	MIC, μg/ml[f] *T. vaginalis*	MIC, μg/ml[f] *E. histolytica*
54	Ph	$CH=CH_2$[d]	33	159-160	$C_{13}H_{11}N_3O_2$	1.0	8.0
55	4-CH_3Ph	$CH=CH_2$	79	137-138	$C_{14}H_{13}N_3O_2$	2.0	16.0
56	4-CH_3Ph	$CH=CHCH_3$	19	147-148	$C_{15}H_{15}N_3O_2$	2.0	32.0
57	3,4-$(CH_3)_2$Ph	$CH=CH_2$	20	136-137	$C_{15}H_{15}N_3O_2$	0.5	16.0
58	2,4,6-$(CH_3)_2$Ph	$CH=CH_2$	84	162-163	$C_{16}H_{17}N_3O_2$	8.0	100-1000
59	2-CH_3Ph	$CH=CH_2$	77	165-166	$C_{14}H_{13}N_3O_2$	1.0	64.0
60	4-*i*-C_3H_7Ph	$CH=CH_2$	83	122-123	$C_{16}H_{17}N_3O_2$	1.0	32.0
61	4-*i*-C_3H_7Ph	$CH=CHCH_3$	19	90-91	$C_{17}H_{19}N_3O_2$	4.0	16.0
62	4-*tert*-C_4H_9Ph	$CH=CH_2$	83	153-154	$C_{17}H_{19}N_3O_2$	0.5	16.0
63	4-C_2H_5Ph	$CH=CH_2$	89	135-136	$C_{15}H_{15}N_3O_2$	1.0	32.0
64	4-C_2H_5Ph	$CH=CHCH_3$	10	115-116	$C_{16}H_{17}N_3O_2$	2.0	10-100
65	4-*n*-C_3H_7Ph	$CH=CH_2$	80	101-102	$C_{16}H_{17}N_3O_2$	4.0	16.0
66	4-*n*-C_4H_9Ph	$CH=CH_2$	74	88-89	$C_{17}H_{19}N_3O_2$	4.0	32.0
67	4-*n*-C_4H_9Ph	$CH=CHCH_3$	14	80-81	$C_{18}H_{21}N_3O_2$	16.0	64.0
68	4-*sec*-C_4H_9Ph	$CH=CH_2$	61	77-78	$C_{17}H_{19}N_3O_2$	4.0	16.0
69	4-*n*-C_8H_{17}Ph	$CH=CH_2$	57	65-66	$C_{21}H_{27}N_3O_2$	>1000	>1000
70	4-*cyclo*-C_6H_{11}Ph	$CH=CH_2$	79	141-142	$C_{19}H_{21}N_3O_2$	4.0	32.0
71	4-PhPh	$CH=CH_2$	87	222-223	$C_{19}H_{15}N_3O_2$	2.0	100-1000
72	1-Naphthyl	$CH=CH_2$	88	174-175	$C_{17}H_{13}N_3O_2$	2.0	100-1000
73	2-Naphthyl	$CH=CH_2$	95	212-213	$C_{17}H_{13}N_3O_2$	4.0	16.0
74	2-ClPh	$CH=CH_2$	78[b]	203-204	$C_{13}H_{10}ClN_3O_2$	4.0	64.0
75	4-PhPh	$CH=CH_2$	52	154-155	$C_{13}H_{10}ClN_3O_2$	2.0	16.0
76	3,4-Cl_2Ph	$CH=CH_2$[e]	10	138	$C_{13}H_9Cl_2N_3O_2$	2.0	32.0
77	2-MeOPh	$CH=CH_2$	72	178-179	$C_{14}H_{13}N_3O_3$	0.25	16.0
78	3-MeOPh	$CH=CH_2$	83	138-139	$C_{14}H_{13}N_3O_3$	0.5	64.0
79	4-MeOPh	$CH=CH_2$	13	155-156	$C_{14}H_{13}N_3O_3$	1.0	32.0
80	3,4,5-$(MeO)_3$Ph	$CH=CH_2$	87	165-166	$C_{16}H_{17}N_3O_5$	0.5	32.0

[a]All compds were prepd by method G and crystd from EtOH except where noted. [b]Crystd from acetone. [c]All compds were analyzed for C, H, N. [d,e]Prepd by methods D and E, respectively. [f]Results were obtained by serial diln.

Table V



Compd	R	Yield, %[a]	Mp, °C	Formula[b]	MIC, μg/ml[c] *T. vaginalis*	MIC, μg/ml[c] *E. histolytica*
81	CH_3	23	179-180	$C_{14}H_{11}N_3O_2$	8.0	128.0
82	$CH(CH_3)_2$	32	134-135	$C_{16}H_{15}N_3O_2$	4.0	64.0
83	C_2H_5	34	151-152	$C_{15}H_{13}N_3O_2$	64.0	100-1000

[a]All compds were crystd from EtOH. [b]Analyzed for C, H, N. [c]Obtained by serial diln.

Table VI

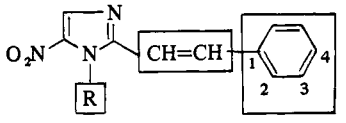
Compd	% activity[a] *T. vaginalis*, mice, 20 mg/kg × 5 po	% activity[a] *E. histolytica* Rats (intest), 100 mg/kg × 5 po	% activity[a] *E. histolytica* Hamsters (hepatic), 100 mg/kg × 5 ip
50	65	Inactive	83
55	50	100	100
58	60	50	Inactive
60	55	Inactive	100
65	90	100	67
68	68	100	100
81	83	Inactive	75
82	83	Inactive	79
83	66	Inactive	67
Metronidazole	100	100 (25 mg/kg × 5 po)	100 (25 mg/kg × 5 po)
2			

[a]*T. vaginalis.* Per cent activity is calculated from the extent of visible diminution of diffuse visceral lesions together with reduction of parasites present in lesions. *E. histolytica* Rat. Per cent activity is calculated from the extent of visible reduction of pathological change in the caecum together with diminution of parasites present in the caecal lesions. *E. histolytica* Hamster. Per cent activity is calculated from the extent of diminution of liver necrosis together with the reduction of parasites present in the necrotic tissue. In these tests normal and infected controls were included.

Table VII. Minimum Dose Level in mg/kg 100% Effective against *Trypanosomal* Infections in Mice

	Tryp. rhodiesiense[a]		*Tryp. cruzi*[b]		*Tryp. gambiense*[a]	*Tryp. congolense*[a]
Compd	Ip	Po	Ip	Po	Ip	Ip
48	200	Inactive		Inactive	N. D.	N. D.
49	>200	Inactive		Inactive	N. D.	N. D.
50	200	Inactive	200	Inactive	N. D.	N. D.
51	200	Inactive		Inactive	N. D.	N. D.
53	100	Inactive		Inactive	50	200
54	>500	Inactive		Inactive	>500	>500
55	50	200	200	500	25	100
56	100	200	500	Inactive	25	50
57	>500	Inactive		Inactive	N. D.	N. D.
60	200	Inactive	200	Inactive	100	100
61	100	200	>500	>500	200	200
63	200	200	200	Inactive	50	200
64	100	Inactive		Inactive	50	>100
65	50	100	100	>500	25	100
66	50	Inactive	200	>500	100	25
67	50	500		Inactive	N. D.	N. D.
68	50	100	100	200	50	100
69	500	>200		Inactive	N. D.	N. D.
70	200	>500	200	Inactive	50	100
81	>500	Inactive			>200	>200
82	500	Inactive	500	Inactive	200	>200
Suramin	1	Inactive	Inactive		5	Inactive at 5
Pentamidine	1.25	Inactive	Inactive		5.0	>5
Diminazene	1	Inactive	>10		5.0	10
Melarsoprol	0.75	0,5	Inactive		0.75	Inactive

[a]Mice were dosed for four consecutive days, commencing on the day of infection. 100% efficacy is equivalent to 30-day postinfection 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 postinfection survival with negative parasitemia. N. D. = Not done.



A B C

following criteria were necessary. (1) The benzene ring should be substituted by a small 4-alkyl group which itself should carry a hydrogen atom on the α-carbon atom. (2) The linkage between the nitroimidazole and the aryl function should be olefinic. (3) The nitroimidazole should carry an *N*-vinyl substituent.

Although none of the compounds reported in this paper approaches the absolute antitrypanosomal activity of pentamidine, suramin, diminazine, or melarsoprol, nevertheless they represent a new class of compounds having broad spectrum antiparasitic activity.

Experimental Section

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

1-(2-Hydroxyethyl)-5-nitro-2-(4-isopropylstyryl)imidazole. Method A (17). A soln of 10.5 g (0.45 g-atom) of Na in 150 ml of MeOH was added rapidly to a stirred soln of 51.1 g (0.3 mole) of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and 60 ml (*ca.* 0.4 mole) of 4-isopropylbenzaldehyde in 500 ml of EtOH at 70°. Stirring was contd for about 0.5 hr at 70°. The dark brown reaction soln was cooled to room temp and dild with an equal vol of H_2O, and the crystals were filtered. Recrystn from EtOH gave 19.1 g (21%) of product, mp 171–172°.

1-(2-Hydroxyethyl)-5-nitro-2-(4-methylstyryl)imidazole. Method B (13). A soln of 21 g (0.9 g-atom) of Na in 300 ml of MeOH was added rapidly to a stirred soln of 103 g (0.6 mole) of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and 100 ml (*ca.* 0.8 mole) of 4-tolualdehyde in 300 ml of DMSO at room temp. After standing overnight, the dark brown soln was stirred and carefully dild with 800 ml of H_2O. Filtration and washing of the crystals with two 1-l. portions of EtOH–H_2O (1 : 1 v/v) gave 125.5 g (77%) of product, mp 143–144°.

Tosylates. General Method C. The compound prepd as by method A or B (0.1 mole) was dissolved in a min vol of dry C_5H_5N and cooled in ice bath, and TsCl (0.11 mole) was added gradually as a solid or a soln in a min vol of C_5H_5N with stirring. The soln was left overnight at room temp. Where a crystal mass formed the solid was filtered off and recrystd from EtOH. Where no crystals formed, the soln was poured with stirring onto ice–H_2O (3 l.). The solid was filtered off and recrystd from EtOH to yield the desired tosylate.

5-Nitro-2-styryl-1-vinylimidazole. Method D (54). 2-Methyl-5-nitro-1-vinylimidazole (5 g, 0.032 mole) and benzaldehyde (5 ml, *ca.* 0.05 mole) were dissolved in 50 ml of EtOH, and 3 ml of 4 *N* NaOH soln was added. The brownish reaction soln was left at room temp for 3 hr with occasional shaking. A crystn solid pptd which was filtered and washed with cold EtOH to yield 5.3 g of hydrated product, 2-(2′-hydroxy-2′-phenyl)ethyl-5-nitro-1-vinylimidazole, mp 165–166°. This latter compd (16.5 g, 0.064 mole) was refluxed for 1 hr with 80 ml of Ac_2O. The soln was poured onto 300 g of ice and the pptd solid was filtered, washed with H_2O, and recrystd from EtOH to give 7.4 g (33%) of product, mp 159–160°.

2-(3,4-Dichlorostyryl)-5-nitro-1-vinylimidazole. Method E (76). 2-Methyl-5-nitro-1-vinylimidazole (15.3 g, 0.1 mole) and 3,4-dichlorobenzaldehyde (20 ml, 0.15 mole) were dissolved in 100 ml of EtOH, and 9 ml of 4 *N* NaOH soln was added. The soln was gently refluxed for 3 hr. The dark brown soln was cooled at 0° overnight, and the crystals were filtered off and washed with cold EtOH. Recrystn from EtOH (carbon) gave 3.2 g (10%) of product, mp 138°.

2-(3,4-Dimethylstyryl)-5-nitro-1-vinylimidazole. Method F (57). A soln of 3.5 g (0.15 g-atom) of Na in 60 ml of MeOH was added rapidly to a stirred soln of 15.3 g (0.1 mole) of 2-methyl-5-nitro-1-vinylimidazole and 20 ml (0.15 mole) of 3,4-dimethylbenzaldehyde in 100 ml of EtOH at 70°. Stirring was contd for 0.5 hr at 70°. The dark brown soln was cooled at 0° for several hours, and the crystals were filtered off. Recrystn from EtOH gave 2.7 g (20%) of product, mp 136–137°.

2-(4′-Methylstyryl)-5-nitro-1-vinylimidazole. Method G (55). A soln of 1.25 g (0.054 g-atom) of Na in 45 ml of EtOH was added rapidly to a stirred suspension of 23 g (0.054 mole) of tosylate (24) in 300 ml of EtOH at 70°. Stirring was contd for 0.5 hr at 70°. After cooling and standing overnight at room temp, the yellow solid was filtered off and thoroughly washed with H_2O. Recrystn from EtOH gave 10.9 g (79%) of product, mp 137–138°.

2-(4′-Methylstyryl)-5-nitro-1-propenylimidazole (56). 1-(2-Hydroxy-*n*-propyl)-2-methyl-5-nitroimidazole (18.5 g, 0.1 mole), prepared as in ref 19, was allowed to react with 4-tolualdehyde *via*

method B. The crude bright yellow cryst solid (12.5 g, 44%) was converted *via* method C to the tosylate (9.0 g, 58%), which in turn was allowed to react *via* method G to give 4.1 g (73%) of required product, mp 147–148°.

1-(2-Chloroethyl)-2-(4-methylstyryl)-5-nitroimidazole (53). Compd 13 (27.3 g, 0.1 mole) was stirred in 60 ml of DMF at room temp. $SOCl_2$ (7.5 ml, >0.1 mole) was added dropwise to the clear soln. A crystal mass formed after *ca.* 5 min, and, after standing for 1 hr, the crystals were filtered, washed with C_6H_6, and dried to give 22.5 g (77%) of product, mp 159–160°.

2-(4-Methylstyryl)-1-(2-morpholinoethyl)-5-nitroimidazole (52). Tosylate 31 (21.4 g, 0.05 mole) and 50 ml of morpholine were warmed on the steam bath for 2 hr. The dark brown soln was cooled and dild with 50 ml of C_6H_6, followed by 200 ml of Et_2O pptg out morpholine tosylate. The supernatant was C treated, evapd (*in vacuo*) to low vol, and treated with *n*-hexane to dissolve out morpholine. The resultant oily solid was dissolved in C_6H_6–Et_2O (75:25 v/v) and chromatographed on a silica gel column (500 g) ultimately yielding a yellow solid. Crystn from EtOAc–Et_2O gave 1.5 g (9%) of product, mp 125–126°.

5-Nitro-2-(4-tolylethynylene)-1-vinylimidazole (81). Tosylate 31 (71.3 g, 0.17 mole) was stirred in 500 ml of CCl_4, and 9.5 ml of bromine was added dropwise. The bromine color gradually faded, and the mixture was refluxed gently for 1 hr. After standing overnight at room temp, the cream-colored crystals were filtered to give 88.0 g (90%) of the required dibromo compound, mp 156–157°. A suspension of this dibromo compound (29.4 g, 0.05 mole) was stirred in 150 ml of DMSO. DBN (12.4 g, 0.1 mole) was added dropwise to the suspension maintaining the temp at 25–30°. The mixture was heated to 80° and maintained for 3 hr. The yellow-brown soln was cooled and added dropwise with stirring to 750 ml of ice H_2O. The cryst ppt which formed initially soon darkened and became sticky. The supernatant was decanted, and the residual solid was dissolved in $CHCl_3$, C treated, and evapd to give an oil which was extracted repeatedly with petr ether (60–80°) to give a bright yellow cryst solid (13.2 g, 61%) which was treated *via* method G to give 3.8 g (43%) of the required acetylenic product, mp 179–180°.

2-Methyl-5-nitro-1-vinylimidazole (5, R = $CH{=}CH_2$). 1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole (50 g, 0.29 mole) was treated by method C to yield 86.1 g (91%) of the tosylate, mp 153°, which was treated as in method G. After cooling to room temp for several hours, the dark brown reaction mix was filtered, and the filtrate was evapd to a vol of *ca.* 100 ml. The concentrate was dild with 500 ml of H_2O and extd with Et_2O (four 250-ml portions). The combined exts were C treated, dried over Na_2SO_4, and evapd to a vol of *ca.* 100 ml, and *n*-hexane was added carefully to ppt a reddish oil which was discarded. The extract was further dild with *n*-hexane to ppt 22.9 g (55%) of the desired product as pale yellow needles, mp 49–50°.

1-Ethyl-2-methyl-5-nitroimidazole (5, R = Et). A suspension of 127 g (1.0 mole) of 2-methyl-5-nitroimidazole in 200 ml of DMF containing 165 g of $(C_2H_5)_2SO_4$ (1.07 moles) was stirred and heated on a steam bath for 3 hr. The DMF was evapd *in vacuo*, and the residue was poured into H_2O when solid (starting material) sepd. This was filtered off, and the filtrate was brought to pH 5 when further solid separated. This was filtered off and added to first solid (total 50 g). The filtrate was basified with solid $NaHCO_3$ and extd with $CHCl_3$ (five 200-ml portions). The $CHCl_3$ extract was dried over $MgSO_4$, the solvent removed *in vacuo*, and the oily residue distd *in vacuo* to give 37.5 g (24%) of the required product, bp 110–112° (1.0 mm). This was identical (ir, nmr) with material prepared by the literature method.[10]

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References

(1) K. Maeda, T. Osato, and H. Umezawa, *J. Antibiot.*, 6A, 182 (1953).
(2) S. Nakamura, *Pharm. Bull.*, 3, 379 (1955).
(3) H. Horie, *J. Antibiot.*, 9, 168 (1956).
(4) C. Cosar, T. Jolon, and M. Bonazet, *Ann. Inst. Pasteur*, 96, 238 (1959).
(5) Antiparasitic Section, *Annu. Rep. Med. Chem.* (1965), and thereafter.
(6) G. P. Ellis, C. Epstein, C. Fitzmaurice, L. Goldberg, and G. H. Lord, *J. Pharm. Pharmacol.*, 16, 801 (1964).
(7) P. N. Giraldi, V. Mariotti, and I. de Carneri, *J. Med. Chem.*, 11, 66 (1968).
(8) Merck and Co., Inc., U. S. Patent 3,472,864 (Oct 14, 1969); Merck and Co., Inc., French Patent 1,423,701 (Aug 6, 1964).
(9) A. F. Cockerill, R. C. Harden, and D. N. B. Mallen, *J. Chem. Soc., Perkin Trans. 2*, in press.
(10) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitisheff, and R. Vanpre, *Arzneim.-Forsch.*, 16, 23 (1966).
(11) K. Eiter and H. Oediger, *Justus Liebigs Ann. Chem.*, 682, 62 (1965).
(12) G. Kobrich and P. Buck in "Chemistry of Acetylenes," H. G. Viehe, Ed., Marcel Dekker, New York, N. Y., 1969, pp 100–101.
(13) H. C. Brown and G. Zweifel, *J. Amer. Chem. Soc.*, 83, 3834 (1961).
(14) M. Honigberg, *J. Parasitol.*, 47, 545 (1961).
(15) A. C. Cuckler, C. M. Malanga, and J. Conroy, *Amer. J. Trop. Med. Hyg.*, 19, 916 (1970).
(16) W. R. Jones, *Ann. Trop. Med. Parasitol.*, 40, 130 (1946).
(17) J. W. Reinertson and P. E. Thompson, *Proc. Soc. Exp. Biol. Med.*, 76, 518 (1951).
(18) F. Hawking, *Exp. Chemother.*, 1, Chapter 5 (1963).
(19) Rhone-Ponlenc S. A., Eire Patent 1058/64 (Oct 12, 1964).

Metabolism of 2-(4-Chlorophenyl)thiazol-4-ylacetic Acid (Fenclozic Acid) and Related Compounds by Microorganisms

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Eleven metabolites produced by microorganisms from the antiinflammatory agent fenclozic acid (1) differed from those produced by mammals. There was no overlap. Microorganisms preferred to attack the acetic acid side chain whereas mammals hydroxylated the 4-chlorophenyl ring. The alcohol metabolite (3) had similar antiinflammatory activity to fenclozic acid, and the amides 10–13 showed an interesting level of potency, but no metabolite was more potent. A novel metabolic α-hydroxylation of the alcohol 3 has been shown to occur with 100% stereospecificity.

2-(4-Chlorophenyl)thiazol-4-ylacetic acid (1) (fenclozic acid) is a potent antiinflammatory agent in rats, mice, and guinea pigs.[1-3] It has been evaluated in patients with rheumatoid arthritis, but was withdrawn when it was found to produce cholestatic jaundice in patients receiving a high initial dose.[4] The action of microorganisms on fenclozic acid has been studied in order to compare the metabolites with those produced by mammals.[5] Desired metabolites