of a clear oil: bp 165–170 °C (0.04 mm); NMR ($CDCl_3$) δ 1.1–1.6 (m, 12 H, OCH_2CH_3), 2.8–3.2 [m, 9 H, $CH_3CH_2O(O)CCH_2CHC(O)OCH_2CH_3$, $P(O)OCH_2CH_3$], ($C_{13}H_{25}O_7PS$).

2-[(Phosphonomethyl)thio]succinic Acid (11). To ester **22** (2.0 g, 5.6 mmol) was added 6 N HCl (35 mL), and the solution was refluxed for 36 h. After treatment with charcoal (Norit A), water was removed in vacuo, leaving a thick oil that over 3 days in vacuo solidified to give 1.0 g (73%) of a white solid: mp 140–141 °C; (D_2O) δ 2.6–2.8 (d, 2 H, J_{H-P} = 13 Hz, SCH_2P), 2.6–2.7 [m, 2 H, $DOOCH_2CH(COOD)S$], 3.5–3.8 [t, 1 H, J = 6 Hz, $DOOCCH_2CH(COOD)$]. Anal. ($C_5H_9O_7PS$) C, H.

Dibenzyl *N*-[[(Diphenylphosphono)methyl]sulfonyl]aspartate (25). The *p*-toluenesulfonic acid salt of dibenzyl L-aspartate was converted to the free amine **23** by dissolving the salt in 150 mL of water and adjusting the pH to above 9.0 with a saturated solution of Na_2CO_3. This solution was then extracted with ether (2 × 150 mL), and the combined ether fractions were dried over Na_2SO_4. Diphenyl [(phenoxysulfonyl)methyl]-phosphonate (**24**), synthesized by the method of Fild and Riech,[15] (2.06 g, 5.1 mmol) was combined with the free base form of dibenzyl L-aspartate (**23**; 1.6 g, 5.1 mmol) in 20 mL of toluene and refluxed for 16 h. The products were applied to a silica gel column and separated by elution with EtOAc/hexanes (1:3). After removal of solvent, the product was crystallized from EtOAc/hexane to give 1.6 g (44%) of white crystals: mp 72–73 °C; NMR ($CDCl_3$) δ 2.8–3.1 (m, 2 H, CH_2COOBz), 4.2 (d, 2 H, J_{H-P} = 13 Hz, SCH_2P), 4.6–4.8 [m, 1 H, $(BzOOC)CHNHS(O_2)CH_2COOBz$], 5.5 (d, 4 H, J = 2 Hz, $COOCH_2Ph$), 6.3–6.5 [m, 1 H, $NHS(O)CH_2P$], 7.1–7.5 (m, 20 H, aromatic H); mass spectrum, *m/e* 623.1366 (M^+), ($C_{31}H_{30}NO_9PS$).

***N*-[(Phosphonomethyl)sulfonyl]aspartic Acid (10).** Tetraester **25** (0.73 g, 1.2 mmol) was refluxed for 4.5 h in 10% NaOH (20 mL). After extraction with ether (20 mL), the aqueous solution was passed through a Dowex 50-X8 column (1.5 × 40 cm, H^+ form, 100–200 mesh) and the product eluted with water. The acidic fractions were pooled and evaporated to dryness in vacuo. The solid residue was dissolved in acetone, to which cyclohexylamine (81 μL, 1.1 mmol) was added with vigorous stirring. Filtration of the precipitate gave 0.35 g (72%) of a white solid: dec pt 185–187 °C; NMR (D_2O) δ 2.7–2.8 (m, 2 H, $DOOCCH_2CHCOOD$), 3.5 [2 d, H, J_{H-P} = 15 Hz, $S(O_2)CH_2P$], 4.0–4.3 [q, 1 H, J = 6 Hz, $CH_2CH(COOD)ND$], monocyclohexylamine salt. Anal. ($C_5H_{10}NO_9PS$) C, H.

Dibenzyl *O*-(Bromoacetyl)malate (28). Dibenzyl L-malate[31] (**26**; 6.0 g, 19 mmol) and triethylamine (1.92 g, 19.2 mmol) were combined in ether (25 mL) at room temperature, and bromoacetyl chloride (**27**; 3.0 g, 19.2 mmol) in ether (50 mL) was added dropwise over 20 min. The products were filtered to remove triethylamine hydrochloride, and solvent was removed in vacuo. The residue was column chromatographed on silica gel with petroleum ether/ether (3:1) to give 3.2 g (38.5%) of a clear oil: NMR ($CDCl_3$) δ 2.95 (d, 2 H, J = 6 Hz, CH_2COOBz), 3.8 [s, 2 H, $C(O)CH_2Br$], 5.2–5.3 (d, 4 H, J = 2 Hz, OCH_2Ph), 5.6 (t, 1 H, J = 6 Hz, $CHCOOBz$], 7.3 (s, 10 H, aromatic H), ($C_{20}H_{19}BrO_6$).

Dibenzyl *O*-[(Dimethylphosphono)acetyl]malate (29). To the bromide **28** (3.2 g, 7.3 mmol) was added trimethyl phosphite (1.1 g, 8.6 mmol) and the mixture rapidly heated to reflux for 1 h. Products were purified by column chromatography on silica gel, eluted with hexane/EtOAc (3:7), to give 2.1 g (60%) of a clear oil: NMR ($CDCl_3$) δ 2.9 (d, 2 H, J = 6 Hz, CH_2COOBz), 3.1 [d, 2 H, J_{H-P} = 12 Hz, $C(O)CH_2P(O)(OCH_3)_2$], 3.7 [d, 6 H, J_{H-P} = 12 Hz, $P(O)(OCH_3)_2$], 5.2 (d, 4 H J = 2 Hz, OCH_2Ph), 5.6 (t, 1 H, J = 6 Hz, $CHCOOBz$), 7.3 (s, 10 H, aromatic H); mass spectrum, *m/e* 464.1220 ($C_{22}H_{25}O_9P$).

Dibenzyl *O*-(Phosphonoacetyl)malate (30). The ester **29** (1.0 g, 2.2 mM) was combined with bromotrimethylsilane (0.78 mL, 5.9 mM) in 10 mL of chloroform and the reaction stirred for 20 min. The solvents and volatile constituents were removed on the rotoevaporator, the remainder was diluted with 25 mL of ether, treated with charcoal Norit A, and filtered. Water (0.2 mL) was then added, the ether was removed on the rotoevaporator, and an additional 10 mL of water was added. The pH of the solution was adjusted to 7.0 with 0.1 N NaOH; lyophilization of the water gave 0.61 g (79%) of a solid: dec pt 230–232 °C; NMR (D_2O) δ 2.6–3.1 [m, 4 H, $BzO(O)CCH_2C(H)OC(O)CH_2P$], 5.1 (d, 4 H J = 2 Hz, OCH_2Ph), 5.35 (t, 1 H, J = 6 Hz, $BzOOCCH_2CHCOOBz$), 7.2 (s, 10 H, aromatic H), ($C_{20}H_{19}Na_2O_9P$).

***O*-(Phosphonoacetyl)-L-malic Acid (6).** The benzyl ester **30** (0.86 g, 1.8 mmol) was dissolved in water (25 mL), and 0.3 g of 10% Pd on charcoal was added. The solution was hydrogenated for 45 min on a Parr apparatus under 45 psi of H_2. The solution was filtered, the pH adjusted to 7.0 with 0.1 N NaOH, and the solution lyophilized to yield 0.5 g (83%) of a crystalline solid: dec pt 250–252 °C; NMR (D_2O) δ 2.7–3.2 [m, 4 H, $CH_2CHOC(O)CH_2P$], 5.0 (s, HDO), 5.0–5.3 (t, 1 H, $CH_2CHCOOD$). Anal. ($C_6H_5Na_4O_9P$) C, H, P. For mass spectral analysis, **6** was derivatized with diazomethane to give the corresponding tetramethyl esters. Mass spectrum: *m/e* found 312.0609 (M^+).

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Registry No. **6**, 97998-19-1; **7**, 97998-20-4; **8**, 97998-21-5; **9**, 97998-22-6; **10**, 98013-92-4; **11**, 97998-23-7; **12**, 97998-24-8; **13**, 97998-25-9; **14**, 31618-90-3; **15**, 97998-26-0; **16**, 97998-27-1; **17**, 54820-26-7; **18**, 70660-08-1; **19**, 70660-05-8; **20**, 2409-52-1; **21**, 623-91-6; **22**, 97998-28-2; **23**, 2791-79-9; **24**, 73300-72-8; **25**, 97998-29-3; **26**, 85719-40-0; **27**, 22118-09-8; **28**, 97998-30-6; **29**, 97998-31-7; **30**, 97998-32-8; *p*-toluenesulfonyl chloride, 98-59-9; diethyl (hydroxymethyl)phosphonate, 3084-40-0; triethyl phosphite, 122-52-1; trimethyl phosphite, 121-45-9; aspartate transcarbamylase, 9027-25-2.

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Antitrichomonal Activity of Mesoionic Thiazolo[3,2-*a*]pyridines[1]

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Screening of mesoionic compounds as potential electron acceptors by analogy with metronidazole led to the finding of in vitro antitrichomonal activity for anhydro-2-phenyl-3-hydroxythiazolo[3,2-*a*]pyridinium hydroxide (1). In a series of analogues, potent in vitro activity was found to be associated with amino substitution; however, such activity was dependent on specific structural features and not on the reduction potential. The most active compounds showed only poor in vivo activity.

For many years the chemotherapy of trichomonal (*Trichomonas vaginalis*) infections has been dominated by the nitroheterocycles, particularly in more recent times by the nitroimidazoles. Studies on metronidazole[3-5] (I) and

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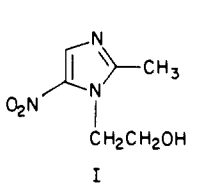
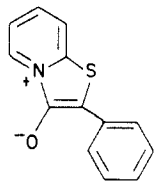


I



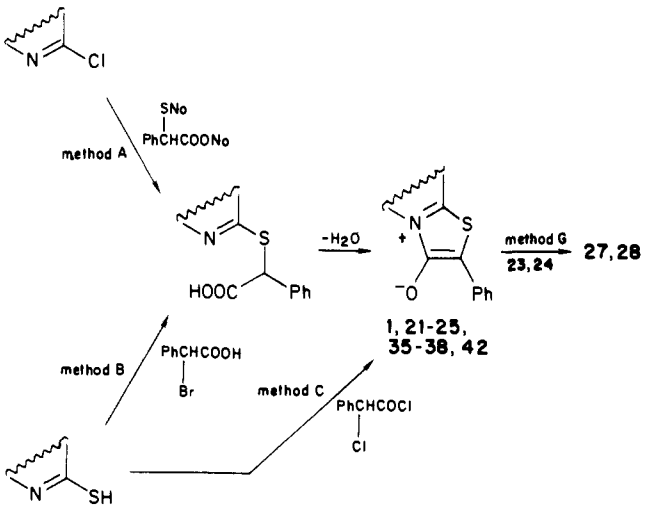
1

related compounds[4] have shown that the remarkable specificity toward anaerobes (and facultative anaerobes) is due to the higher reduction potentials occurring in these cells relative to aerobic (e.g., mammalian) cells. In susceptible cells, reduction of metronidazole establishes a concentration gradient[6,7] favoring further diffusion into these cells, with the accumulation of reduction products. It has been shown that, during the reduction, metronidazole and related nitroimidazoles accept electrons in a four-electron process[8] from an electron-transfer protein (possibly ferredoxin[4,9]), thereby inhibiting hydrogen evolution resulting from anaerobic respiration. The lethal event however is believed to involve the interaction of the reduction products, possibly a hydroxylamine,[6,8] with DNA.[6,10,11]

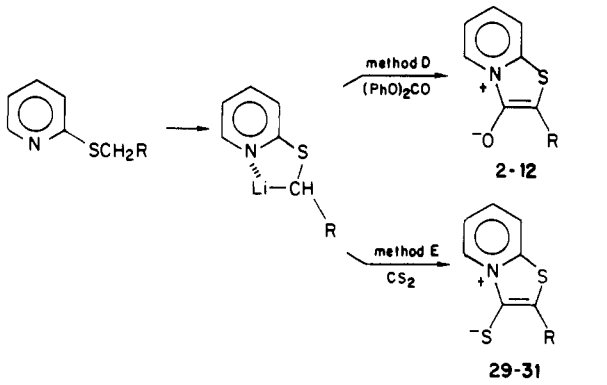
Recently the safety of this class of compounds has been questioned following the finding of potent mutagenicity in the Ames test[8,12-14] and possibly in mice.[15] Although other workers have since argued against the significance of these findings,[16,17] it was this uncertainty that prompted our efforts to find alternative compounds.

Since reduction of the nitro group is a prerequisite for activity,[11] its replacement by other electron-withdrawing[18] or reducible groups[19] as attempted for the related nitrofurans seemed doomed to fail. We reasoned that a more fruitful approach might be the screening of other readily reducible organic molecules and initiated a program of screening in conjunction with the measurement of redox potentials, using the reduction potential of metronidazole as the standard. Mesoionic compounds were considered to be a promising starting point since the formal positive charge in the ring should enhance electron addition; yet,

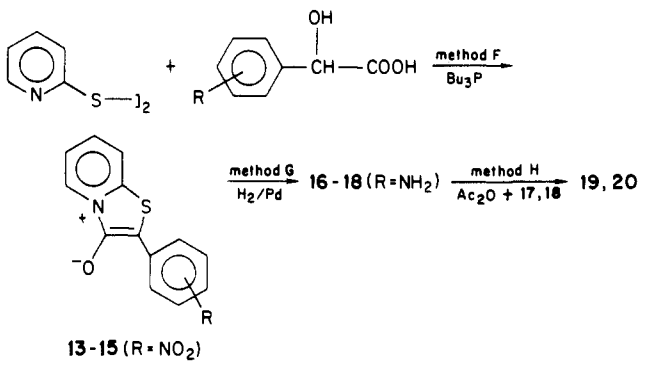
Scheme I



Scheme II



Scheme III



the absence of a net charge in the molecule would be more favorable for passage across membranes. Testing of a variety of ring systems against *Tritrichomonas foetus* eventually led to the finding of potent in vitro activity in **1**, and the elaboration of this lead is reported here.

Chemistry

The parent compound **1**, as well as **21–25**, **35–38**, and **42**, were synthesized by methods A–C using standard cyclization conditions[20-23] (Scheme I). In some cases it was

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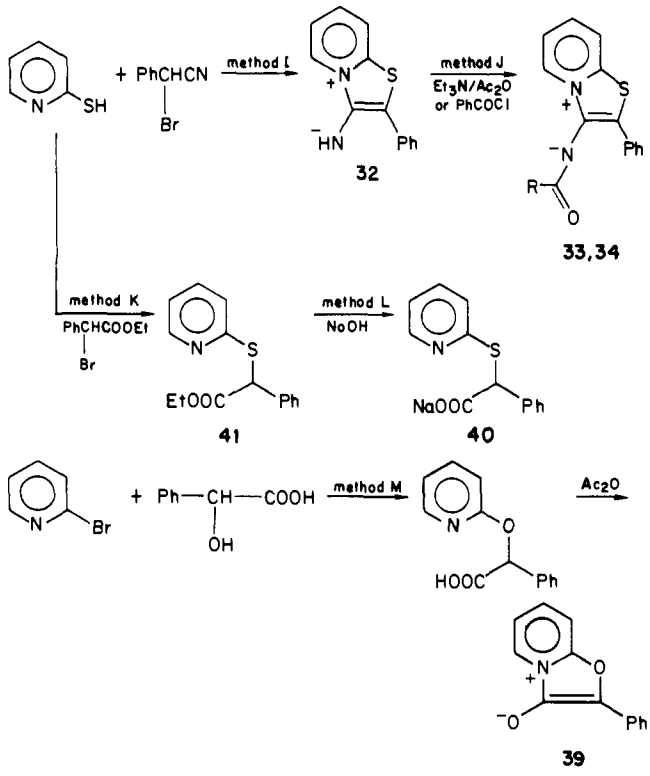
Scheme IV



found more convenient to prepare the intermediate acid with (mercaptophenyl)acetic acid (method A). No dehydration step was required for **25**, which further gave **26** on treatment with Ac_2O. These methods, however, were found to be unsuitable for the efficient preparation of analogues in which the phenyl ring of **1** was substituted or replaced. The synthesis of the required α-halo acids was somewhat lengthy; in addition, several of the acids needed were unstable. These factors prompted us to devise two new routes to the mesoionic thiazolo[3,2-*a*]pyridinium ring system, the first of which is shown in Scheme II. The appropriate thioethers, available from 2-mercaptopyridine and the corresponding chloromethyl compounds, were treated with *n*-butyllithium or LDA to give highly colored anions. When the mixture was quenched with diphenyl carbonate (method D), compounds **2–12** were obtained, while trapping with carbon disulfide (method E) afforded the sulfur analogues **29–31**. The second method (Scheme III) employed the readily available mandelic acids as precursors. Compounds **13–15** were formed directly when the acids were treated with 2,2′-dipyridyl disulfide and tri-*n*-butylphosphine.

Hydrogenation of **13–15** and of **23** and **24** gave **16–18** and **27** and **28** from which **19** and **20** were obtained routinely. Compound **32** was obtained directly from the reaction with α-bromophenylacetonitrile (Scheme IV) and converted to the acyl derivatives **33** and **34**, whereas the oxazole analogue **39**, representing a new ring system, was obtained from 2-bromopyridine (method M). The open-chain compounds **40** and **41** were obtained routinely from 2-bromo- and 2-mercaptopyridine.

Biological and Physical Methods

Antitrichomonal activity was determined with *T. foetus* as the test organism since it is easier to handle and gives more reproducible results than *T. vaginalis*. All compounds were evaluated for in vitro activity by using a broth microdilution assay [Experimental Section]. In vivo experiments were conducted by using a vaginal infection in Syrian hamsters.[24] Test compounds were simultaneously administered both vaginally as a 2% formulation in an aqueous propylene glycol cream[25] (once daily) and subcutaneously as a suspension[26,27] (b.i.d.), for 4 or 8 days, with metronidazole as the positive control. Reduction potentials were determined by differential-pulse polarography in dimethylformamide (or mixtures thereof with water) and are expressed as peak voltage values (E_p) relative to a saturated calomel electrode.

Discussion—Structure–Activity Relationships

Examination of the data presented in Table I shows that among the compounds tested, in vitro antitrichomonal activity is found only in compounds retaining the basic ring system of **1**, i.e. anhydro-2-aryl-3-hydroxythiazolo[3,2-*a*]-pyridinium hydroxides. Replacement of the thiazole sulfur by oxygen (**39**) or even replacement of the exocyclic oxygen by sulfur (**29–31**) or nitrogen (imino **32** or imido **33** or **34**) is sufficient to completely destroy activity at the levels tested. Substitution of the fused pyridine ring (**21–28**) is poorly tolerated (with the notable exception of **24**), and its replacement by other fused heterocycles (**35–38**) or phenyl (**42**) also renders the original lead inactive. However, substitution of the 2-phenyl ring (**2–6**; **13–20**) or its replacement by pyridyl (**9–11**) is generally well tolerated (with the exception of **14**, **15**, **19**, and **20**), though the aryl group must be attached by a direct bond (**8**, **9**). The presence of an amino substituent at the 4-position of the phenyl ring causes a dramatic increase in activity (**6**, **18**), otherwise the in vitro activity seems to be relatively insensitive to the substituent except for the 3- or 4-nitro or acetamido groups where it is diminished. The marked activity of the 8-nitro compound **24** relative to the 6-nitro derivative **23** is surprising, yet fully reproducible. The inactivity of the open-chain compounds **40** and **41** shows that the antitrichomonal activity of **1** is not due to hydrolysis products.

Particularly surprising is the lack of any correlation of activity with reduction potential. For reasons of solubility, reduction potentials were measured in DMF, in which metronidazole gave a value of −1.15 V. However, although the measured value for metronidazole becomes less negative with increasing water content of the solution, reaching −0.49 V in pH 6 buffer (lit.[28] −0.49 V), we found that for various mesoionic systems the reduction potential remains constant in aqueous DMF solutions containing between 0 and 50% buffer. Thus, the measured reduction potentials for the active compounds (−1.46 to −1.72 V) are too negative to allow the acceptance of electrons from ferredoxin (lit.[28] reduction potential −0.53 V). Furthermore, more readily reduced compounds such as **36** and **38**, where the reduction potentials approximate that of metronidazole (in DMF), are inactive. The disparity between close analogues such as **1** and **29** cannot be explained by differences in lipophilicity since estimation of the lipophilicity as log K' (pH 7.4) gave values of 1.95 and 1.73, respectively.

Testing of the more active compounds in vivo using the 4-day treatment regimen gave disappointing results (Table I). Most compounds proved to be inactive at each culture time, although the general poor solubility prevented proper distribution from the subcutaneous site in several cases. Compound **12** produced negative washes in 80% of the

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(25) The cream contained stearic acid, Span 60, Span 80, Tween 60, propylene glycol, methyl paraben, propyl paraben, BHA, and water.
(26) The vehicle contained sodium carboxymethylcellulose, sodium chloride, Tween 80, benzyl alcohol, and water.
(27) **40** was administered subcutaneously in water.
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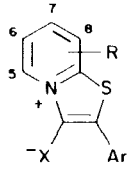
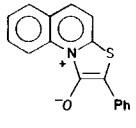
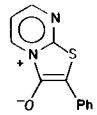
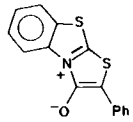
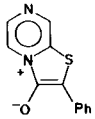
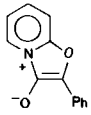
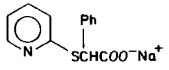
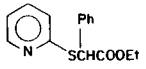
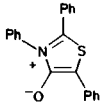
Table I. Physical Properties and Antitrichomonal Activities



compd	Ar	R	X	formula	mp, °C	solvent[a]	anal.[b]	prepn method	MLC,[c] μg/mL	E_p	in vivo vaginal *T. foetus* infection in hamsters[e] day 1	day 4
1	C_6H_5		O	$C_{13}H_9NOS$	172.5–174[f]	C	C, H, N	B	12.5 (12.5)	−1.61	0/5	0/5
2	2-ClC_6H_4		O	$C_{13}H_8ClNOS$	185–187	A	C, H, N	D	100 (25)		0/5	0/5
3	4-ClC_6H_4		O	$C_{13}H_8ClNOS$	225.5–227[d]	A	C, H, N	D	25 (12.5)	−1.56	0/5	0/5[g]
4	4-$CH_3C_6H_4$		O	$C_{14}H_{11}NOS$	193.5–195.5[d]	A	C, H, N	D	12.5 (25)	−1.64	0/5	0/5[g]
5	4-$CH_3OC_6H_4$		O	$C_{14}H_{11}NO_2S$	189.5–190[h]	A	C, H, N	D	6.25/12.5 (12.5)	−1.67	0/5	0/5
6	4-$(CH_3)_2NC_6H_4$		O	$C_{15}H_{14}N_2OS$	234.5–240	A	C, H, N	D	0.8 (12.5)	−1.72	0/5	0/5
7	4-*t*-$C_4H_9C_6H_4$		O	$C_{17}H_{17}NOS$	167–173	C	C, H, N	D	25[i] (6.25)	−1.68		
8	*trans*-$C_6H_5CH{=}CH$		O	$C_{15}H_{11}NOS$	157–160	C	C, H, N	D	200[i] (12.5)			
9	4-ClC_6H_4S		O	$C_{13}H_8ClNOS_2$	194–196[d]	A	C, H, N	D	>200 (25)	−1.57		
10	2-C_5H_4N		O	$C_{12}H_8N_2OS$	201.5–203	A	C, H, N	D	25 (25)	−1.56	1/5	0/5
11	3-C_5H_4N		O	$C_{12}H_8N_2OS$	175–184	A	C, H, N	D	12.5/25 (25/50)	−1.56	0/5	0/5
12	4-C_5H_4N		O	$C_{12}H_8N_2OS$	233–240	A	C, H, N	D	50/100 (25/50)	−1.46	4/5	0/4
13	2-$O_2NC_6H_4$		O	$C_{13}H_8N_2O_3S$	184–185[d]	E	C, H, N	F	25 (12.5)			
14	3-$O_2NC_6H_4$		O	$C_{13}H_8N_2O_3S$	>285	F	C, H, N	F	>200[i] (12.5)			
15	4-$O_2NC_6H_4$		O	$C_{13}H_8N_2O_3S$	>300[j]	E	C, H, N	F	>200[i] (12.5)			
16	2-$H_2NC_6H_4$		O	$C_{13}H_{10}N_2OS$	143–144.5	P	C, H, N	G	3.12/1.6 (12.5)			
17	3-$H_2NC_6H_4$		O	$C_{13}H_{10}N_2OS{\cdot}0.25H_2O$	176–178	H	C, H, N	G	12.5 (3.12)			
18	4-$H_2NC_6H_4$		O	$C_{13}H_{10}N_2OS{\cdot}0.25H_2O$	210–215[k]	H	C, H, N	G	1.6 (12.5)		0/5	0/5
19	3-$AcNHC_6H_4$		O	$C_{15}H_{12}N_2O_2S$	235–237	I	C, H, N	H	200 (12.5)			
20	4-$AcNHC_6H_4$		O	$C_{15}H_{12}N_2O_2S{\cdot}0.25H_2O$	298–300[d,l]	I	C, H, N	H	>200 (12.5)			
21	C_6H_5	5-CH_3	O	$C_{14}H_{11}NOS$	166–167[m,n]	C	C, H, N	C	>200 (12.5)			
22	C_6H_5	7-CH_3	O	$C_{14}H_{11}NOS$	232–235.5[d]	A	C, H, N	C	200 (3.12/6.25)			
23	C_6H_5	6-NO_2	O	$C_{13}H_8N_2O_3S$	284.5–285[d,o]	F	C, H, N	A	200[i] (12.5)			
24	C_6H_5	8-NO_2	O	$C_{13}H_8N_2O_3S$	267.5–268.5[p]	I	C, H, N	A	0.8[i] (12.5)		0/5	0/5
25	C_6H_5	8-OH	O	$C_{13}H_9NO_2S$	269.5–274[d]	F	C, H, N	B[q]	>200 (12.5)			
26	C_6H_5	8-CH_3COO	O	$C_{15}H_{11}NO_3S$	185.5–188.5[r]	C[s]	C, H, N	H[t]	>200 (12.5)			
27	C_6H_5	6-NH_2	O	$C_{13}H_{10}N_2OS{\cdot}0.25H_2O$	216–217	Q	C, H, N	G	100 (12.5)			
28	C_6H_5	8-NH_2	O	$C_{13}H_{10}N_2OS{\cdot}H_2O$	217–219.5	J	C, H, N	G	200[i] (12.5)			
29	C_6H_5		S	$C_{13}H_9NS_2$	196.5–198	B	C, H, N	E	>200 (12.5)	−1.51	0/5	0/5[g]
30	4-ClC_6H_4		S	$C_{13}H_8ClNS_2$	224.5–225.5	B	C, H, N	E	>200 (12.5)			
31	4-$CH_3OC_6H_4$		S	$C_{14}H_{11}NOS_2$	193–197.5[d]	D	C, H, N	E	>200 (12.5)			
32	C_6H_5		NH·HBr	$C_{13}H_{11}BrN_2S$	209–211	G	C, H, N	I	>200 (25/50)	−1.24		
33	C_6H_5			$C_{15}H_{12}N_2OS{\cdot}H_2O$	140–183	K	C, H, N	J	>200 (25/50)	−1.49		
34	C_6H_5			$C_{20}H_{14}N_2OS$	202.5–208.5	K	C, H, N	J	>200 (25/50)			
35				$C_{17}H_{11}NOS$	195–197[u]	M	C, H, N	C	>200 (12.5)			

36		$C_{12}H_8N_2OS$	220–221[v]	C	C, H, N	B	>200 (12.5)	−1.20		
37		$C_{15}H_9NOS_2$	182–186[d,w]	L	C, H, N	B	>200 (12.5)			
38		$C_{12}H_8N_2OS$	227–229	M	C, H, N	A	>200 (12.5)	−1.08		
39		$C_{13}H_9NO_2$	148–153	C	C, H, N	M	>200 (25/50)	−1.70	0/5	0/5
40		$C_{13}H_{10}NNaO_2S{\cdot}0.5H_2O$	221–224[d]	N	C, H, N	L	>200 (25/50)		0/5	0/5
41		$C_{15}H_{15}NO_2S$	oil	H	C, H, N	K	>200 (25/50)		0/5	0/5
42		$C_{21}H_{15}NOS$	250–251[x]	O	C, H, N	B	>200 (25/50)	−1.43		
metronidazole									15/15	15/15

[a] Recrystallization solvents: A, EtOAc–MeOH; B, toluene; C, EtOAc; D, toluene–MeOH; E, THF–isolated directly from reaction mixture; F, DMF; G, EtOH; H, purified by chromatography; I, precipitated with ether; J, MeOH; K, acetone; L, CH_2Cl_2; M, CH_2Cl_2–ether; N, MeOH–acetone; O, CH_2Cl_2–EtOH; P, EtOAc slurry; Q, CH_2Cl_2 slurry. [b] Unless otherwise stated, the analyses are within ±0.4% of the theoretical values. [c] MLC = minimum lethal concentration. Values in parentheses are those for the standard metronidazole in the same assay. Single figures for the MLC represent consistent duplicate end points. [d] With decomposition. [e] Ratio of animals cured/total numbers infected at day 1 and day 4 posttreatment after 4 days of treatment. [f] Literature[20] mp 183–185 °C. [g] Some sc dose remained at autopsy. [h] Literature[21] mp 191–193 °C. [i] Suspension. [j] Literature[21] mp 350 °C. [k] Coalesces. [l] Inserted at 265 °C. [m] Literature[21] mp 166–167 °C. [n] Slow heat, phase change at 157 °C. [o] Inserted at 270 °C. Literature[21] mp 284–290 °C. [p] Literature[21] mp 270–273 °C. [q] No dehydration step needed. [r] Literature[20] mp 182–183 °C. [s] Containing 0.5 mL each of Ac_2O and pyridine. [t] From **25** with Ac_2O/pyridine. [u] Literature[21] mp 198–199 °C. [v] Literature[21] mp 218–220 °C. [w] Literature[22] mp 180 °C dec. [x] Literature[23] mp 251–252 °C.

infected animals on day 1 posttreatment, but 3 days later all animals were again positive.

Compounds **10** and **12** and the more soluble hydrochloride salt of **12** were retested with 10 animals per test compound and an 8-day treatment period. Vaginal washings were taken after 5 and 8 days of treatment and at day 3 posttreatment. Animals treated with **10** remained infected throughout the treatment whereas those treated with **12** produced negative washings in 2/6, 3/4, and 0/2 animals at days 5 and 8 of treatment and on day 3 posttreatment, respectively.[29] This compound proved to be very toxic, killing 80% of the animals before the posttreatment vaginal wash. The hydrochloride salt of **12** killed all the animals before the first vaginal wash.

We have thus shown that the in vitro activity in this series has high structural requirements, specifically the need for a mesoionic 3-hydroxythiazolo[3,2-*a*]pyridinium ring system, although the mechanism of the biological activity is not clear. The (albeit marginal) activity of the 7-substituted compound **22** seems to rule out the possibility of hydride (or nucleophilic) addition to the pyridine ring by analogy with NAD, and it may be that compounds such as **1** act as acylating agents. However, in view of the high toxicity of these compounds and the lack of useful in vivo activity, further investigation of this series was not considered worthwhile.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet spectra were determined in methanol with a Cary 14 instrument. Infrared spectra were obtained in KBr with a Perkin-Elmer 237B spectrometer. NMR spectra were obtained with Varian A-60 and HA-100 instruments, and mass spectra were determined with a Varian-MAT CH4 spectrometer. Elemental analyses were performed by the Analytical Department of Syntex Research, Institute of Organic Chemistry, and are within ±0.4% of calculated values. Reduction potentials were determined relative to a saturated calomel electrode by differential-pulse polarography in DMF (or McIlvains buffer/DMF mixtures) using tetrabutylammonium tetrafluoroborate (0.05 M) as the supporting electrolyte with a Princeton Applied Research Polarographic analyzer, Model 147A.

Method A. Anhydro-2-phenyl-8-nitrothiazolo[3,2-*a*]pyridinium Hydroxide (24). A solution of α-bromophenylacetic acid (3.4 g, 0.016 mol) in acetone (30 mL) was treated with stirring with *n*-butyl potassium xanthate (2.9 g, 0.015 mol). After 10 min, the solution was filtered and the acetone evaporated. The resulting oil was dissolved in 75 mL of MeOH, cooled to 0 °C, and treated with solid NaOH (2.51 g, 0.0627 mol). The resulting solution was stirred overnight, filtered, and added dropwise to a 0 °C solution of 2-chloro-3-nitropyridine (2.5 g, 0.0158 mol) in 40 mL of CH_2Cl_2/MeOH (1:1). After 0.5 h, excess 1.5 N HCl was added and the mixture extracted with CH_2Cl_2. Drying ($MgSO_4$) and evaporation of the solvent afforded an oil that was dissolved in pyridine (25 mL) and treated dropwise with 10 mL of Ac_2O. After 30 min, 20 mL of Et_2O was added and the red solid filtered off and washed with Et_2O to give 1.67 g (42%) of **24**, mp 256–259 °C. Anal. ($C_{13}H_8N_2O_3S$) C, H, N.

Method D. Anhydro-2-(4-chlorophenyl)-3-hydroxythiazolo[3,2-*a*]pyridinium Hydroxide (2). A solution of 2-[(4-chlorobenzyl)thio]pyridine (2.36 g, 0.01 mol) in dry THF (75 mL) at −78 °C was treated dropwise with stirring with *n*-BuLi (1.38 M in hexane) until the red color persisted. An additional 7.25 mL (0.01 mol) of *n*-BuLi solution was then added over 10 min, and the resulting dark red solution was stirred 0.5 h at −78 °C and quenched with diphenyl carbonate (3.2 g, 0.015 mol). The mixture was allowed to warm to room temperature and after a further 1 h at this temperature poured into 500 mL of CH_2Cl_2. The organic layer was washed with 1 N NaOH, water, and brine and dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue from EtOAc–MeOH gave 0.95 g (36%) of **2**, mp 225.5–227 °C dec. Anal. ($C_{13}H_8ClNOS$) C, H, N.

Method E. Anhydro-2-(4-chlorophenyl)-3-mercaptothiazolo[3,2-*a*]pyridinium Hydroxide (30). The solution of anion prepared from 2-[(4-chlorobenzyl)thio]pyridine and *n*-BuLi as in method D was treated at −78 °C with carbon disulfide (3.0 mL, 0.05 mol). After warming to room temperature, the dark red solution was poured into 100 mL of cold 1 N HCl and the mixture stirred for 10 min. The product was extracted with CH_2Cl_2, and the extracts were washed with aqueous K_2CO_3 and water and dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue from toluene/MeOH gave 1.03 g (37%) of **30**, mp 224.5–225.5 °C. Anal. ($C_{13}H_8ClNS_2$) C, H, N.

Method F. Anhydro-2-(3-nitrophenyl)-3-hydroxythiazolo[3,2-*a*]pyridinium Hydroxide (14). A rapidly stirred solution of *m*-nitromandelic acid[30] (1.97 g, 0.01 mol) and 2,2′-dipyridyl disulfide (4.40 g, 0.02 mol) in dry THF (75 mL) was treated with tri-*n*-butylphosphine (5.0 mL, 0.02 mol). The solution instantly became dark red with evolution of heat, and a solid soon precipitated. After the mixture had cooled, the solid was filtered off and washed with THF to give 2.02 g (74%) of **14**, homogeneous by TLC and suitable for further reactions. The analytical sample was recrystallized from DMF, mp >285 °C. Anal. ($C_{13}H_8N_2O_3S$) C, H, N.

Method G. Anhydro-2-(3-aminophenyl)-3-hydroxythiazolo[3,2-*a*]pyridinium Hydroxide (17). A suspension of **14** (0.05 g, 1.84 mmol) in 75 mL of 2-methoxyethanol was hydrogenated (1 atm) over 10% Pd/C (0.5 g). When a sample of the reaction mixture indicated that the starting material had been consumed, the catalyst was removed by filtration through Celite. The solvent was removed by vacuum distillation and the orange-red residue chromatographed on silica gel, eluting with 8% MeOH/CH_2Cl_2 to give 0.29 g (65%) of **17**, mp 176–178 °C. Anal. ($C_{13}H_{10}N_2OS \cdot 0.25H_2O$) C, H, N.

Method H. Anhydro-2-(3-acetamidophenyl)-3-hydroxythiazolo[3,2-*a*]pyridinium Hydroxide (19). A mixture of **17** (0.1 g, 0.0041 mol) and Ac_2O (10 mL) was heated on a hot plate until all the solid dissolved. Hot filtration and addition of Et_2O to the cooled solution gave 0.92 g (78%) of **19** as an orange solid, mp 211–238 °C. Anal. ($C_{15}H_{12}N_2O_2S$) C, H, N.

Method I. 2-Phenyl-3-aminothiazolo[3,2-*a*]pyridinium Bromide (32). A mixture of 2-mercaptopyridine (1.11 g, 0.01 mol) and α-bromophenylacetonitrile (1.96 g, 0.01 mol) in 30 mL of EtOH was heated briefly at reflux. The solution was filtered hot and allowed to cool to give 2.6 g (84%) of yellow crystalline **32**, mp 209–211 °C. Anal. ($C_{13}H_{11}BrN_2S$) C, H, N.

Method J. Anhydro-2-phenyl-3-(benzoylamino)thiazolo[3,2-*a*]pyridinium Hydroxide (34). A stirred slurry of **32** (1.0 g, 3.26 mmol) in 40 mL of CH_2Cl_2 at room temperature was treated with Et_3N (1.32 g, 0.013 mol), followed by benzoyl chloride (1.82 g, 0.013 mol). The solution rapidly became clear and after 10 min was poured into 100 mL of cold 1.5 N NaOH. After stirring for 1 h, the organic layer was separated and the aqueous layer extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated and the resulting yellow solid recrystallized from acetone to give 0.76 g (71%) of **34**, mp 202.5–208.5 °C. Anal. ($C_{20}H_{14}N_2OS$) C, H, N.

33 was prepared in a similar manner using Ac_2O.

Method K. Ethyl 2-Phenyl-2-(2-pyridylthio)acetate (41). Ethyl α-bromophenylacetate (8.5 g, 0.035 mol) and 2-mercaptopyridine (3.0 g, 0.027 mol) were warmed briefly in 20 mL of EtOH and cooled to room temperature. The disappearance of 2-mercaptopyridine was followed by TLC since prolonged heating causes cyclization to **1**. Addition of Et_2O to the solution gave a white solid that was filtered off. Treatment of the solid with aqueous $NaHCO_3$, extraction with Et_2O, and chromatography on silica gel (CH_2Cl_2) gave 6.6 g (87%) of **41**. Anal. ($C_{15}H_{15}NO_2S$) C, H, N.

Method L. Sodium 2-Phenyl-2-(2-pyridylthio)acetate (40). The ester **40** (1.12 g, 0.0041 mol) in 10 mL of MeOH was treated with NaOH (0.164 g, 0.0041 mol) in 1 mL of water. After standing overnight, the solvent was removed and the residue recrystallized

(29) The figures represent the ratio of animals with negative vaginal washings over the total number of surviving animals.

(30) Heller, G. *Chem. Ber.* **1913**, *46*, 3974.

from acetone/MeOH to give a white solid that rapidly absorbed water from the air. The hydrated salt weighed 1.0 g, mp 221–224 °C dec. Anal. ($C_{13}H_{10}NNaO_2S \cdot 0.5H_2O$) C, H, N.

Method M. Anhydro-2-phenyl-3-hydroxyoxazolo[3,2-*a*]pyridinium Hydroxide (39). A rapidly stirred solution of mandelic acid (7.6 g, 0.05 mol) in DMF (50 mL) was treated portionwise with NaH (5.1 g of 50% dispersion in mineral oil, 0.105 mol). When the evolution of hydrogen was complete, 2-bromopyridine (11.9 g, 0.075 mol) was added and the mixture heated at 90 °C for 6 h. Addition of Et_2O to the cooled mixture gave a white solid that was filtered off, washed with Et_2O, and then dissolved in water. Acidification to pH 1 with HCl afforded 6.0 g (52%) of 2-phenyl-2-(2-pyridyloxy)acetic acid as a solid, which was recrystallized from EtOAc/hexane, mp 117–119.5 °C dec. Anal. ($C_{13}H_{11}NO_3$) C, H, N.

This acid (2.5 g, 0.011 mol) in pyridine (3 mL) was treated with 3 mL of Ac_2O for 15 min. Addition of Et_2O gave a red solid that was filtered off and recrystallized from EtOAc to give 1.3 g (56%) of **39**, mp 148–153 °C. Anal. ($C_{13}H_9NO_2$) C, H, N.

Determination of Activity against *T. foetus*. (a) In Vitro. A broth dilution assay using microtiter plates was used to determine the minimum lethal concentration (MLC) of the test compounds. Each compound was serially diluted (200–0.2 μg/mL) in duplicate in Kupferberg's medium[31] (pH 7.2) using a microdiluter. To each well containing 50 μL of diluted compound was added 50 μL of Kupferberg's broth containing 15 parasites/mm^3. Immediately following the addition of the parasites, two drops of sterile mineral oil were placed in each well of the microtiter plate to cover the surface of the growth medium. Plates were then incubated at 30 °C for 48 h after which they were examined microscopically for the presence of viable parasites.

(b) In Vivo Assay. A hamster vaginal infection model[24] was used to determine in vivo activity. Female Golden Syrian hamsters, 2–3 weeks old (~50 g), were infected intravaginally with *T. foetus* recovered from infected reservoir hamsters with Kupferberg's medium. The challenge contained a minimum of 150 parasites/mm^3.

Three days postinfection treatments were begun on groups of five animals per test compound. Each animal received a single intravaginal treatment of 2% (w/w) formulation and two subcutaneous treatments (100 mg/kg) each day for four days.[32] Flagyl, used as a positive control, consistently gave 100% negative vaginal washings using these doses with this treatment schedule. A group of 20 hamsters was treated with placebo. All placebo-treated animals remained infected throughout each experiment.

On day 1 posttreatment and day 4 posttreatment, the vagina of each animal was washed with Kupferberg's medium (0.5 mL) and the washings were examined microscopically for viable parasites.

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Registry No. 1, 32044-03-4; **2**, 97522-57-1; **3**, 97522-58-2; **4**, 97522-59-3; **5**, 43091-16-3; **6**, 97522-60-6; **7**, 97522-61-7; **8**, 97522-62-8; **9**, 97522-63-9; **10**, 97522-64-0; 11, 97522-65-1; **12**, 97522-66-2; **13**, 97522-67-3; **14**, 97522-68-4; **15**, 43091-17-4; **16**, 97522-69-5; **17**, 97522-70-8; **18**, 97522-71-9; **19**, 97522-72-0; **20**, 97522-73-1; **21**, 43091-18-5; **22**, 97522-74-2; **23**, 43091-14-1; **24**, 43091-13-0; **25**, 35143-57-8; **26**, 32002-92-9; **27**, 97522-75-3; **28**, 97522-76-4; **29**, 97522-77-5; **30**, 97522-78-6; **31**, 97522-79-7; **32**, 97522-80-0; **33**, 97522-81-1; **34**, 97522-82-2; **35**, 43091-21-0; **36**, 43091-22-1; **37**, 66085-20-9; **38**, 97522-83-3; **39**, 97522-84-4; **40**, 97522-85-5; **41**, 97522-86-6; **42**, 18100-80-6; $PhCH(Br)CO_2H$, 4870-65-9; $PhCH(SH)CO_2^-Na^+$, 62289-54-7; PhCH(Br)CN, 5798-79-8; $PhCH(Br)CO_2Et$, 2882-19-1; butyl potassium xanthate, 871-58-9; 2-[(4-chlorobenzyl)thio]pyridine, 74032-43-2; *m*-nitromandelic acid, 42164-79-4; 2,2′-dipyridyl disulfide, 2127-03-9; 2-mercaptopyridine, 2637-34-5; mandelic acid, 90-64-2; 2-bromopyridine, 109-04-6; 2-phenyl-2-(2-pyridyloxy)acetic acid, 97522-87-7; 2-chloro-3-nitropyridine, 5470-18-8.

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(32) Compound **24** was tested at 87.5 mg/kg as the subcutaneous dose.

5-(Haloalkyl)-2′-deoxyuridines: A Novel Type of Potent Antiviral Nucleoside Analogue

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Syntheses of 5-(2-haloethyl)-2′-deoxyuridines, 5-(3-chloropropyl)-2′-deoxyuridines, and 5-(2-chloroethyl)-2′-deoxycytidine are described. The antiviral activities of these compounds were determined in cell culture against herpes simplex virus types 1 and 2. All compounds were shown to possess significant and selective antiviral activity. The most potent derivative, 5-(2-chloroethyl)-2′-deoxyuridine (CEDU), inhibited HSV-1 at concentrations below 0.1 μg/mL. It exerted measurable inhibitory effects on cell proliferation only at concentrations higher than 100 μg/mL. In vivo CEDU reduced the mortality rate of HSV-1-infected mice at concentrations lower than 5 mg/kg per day when given intraperitoneally and orally. Thus, it proved to be more effective in this in vivo model than the reference compounds (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and 9-[(2-hydroxyethoxy)methyl]guanine (ACV).

One of the most potent and selective antiviral agents, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) inhibits effectively herpes simplex type 1 virus (HSV-1) and varicella zoster virus (VZV) replication in vitro[1,2] and in vivo[3-7] and shows high promise for the treatment of diseases caused by these viruses.[8-11] Studies of structure–activity relationships of BVDU and related compounds seemed to indicate that optimal anti-HSV-1 activity in cell culture was associated with analogues in which the 5-substituent

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