Mesoionic Purinone Analogs IV: Synthesis and *In Vitro* Antibacterial Properties of Mesoionic Thiazolo[3,2-*a*]pyrimidin-5,7-diones and Mesoionic 1,3,4-Thiadiazolo[3,2-*a*]pyrimidin-5,7-diones

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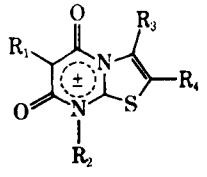
Abstract □ Derivatives of two members of a new and unusual class of heterocycles, termed mesoionic purinone analogs, were synthesized and examined for *in vitro* antibacterial activity. Mesoionic thiazolo[3,2-*a*]pyrimidin-5,7-diones and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5,7-diones, which are isoconjugate with xanthine, were found to exhibit antibacterial activity against both Gram-negative and Gram-positive organisms. Several mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5,7-diones possess significant levels of activity against *Proteus vulgaris* and *Staphylococcus aureus*.

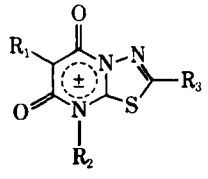
Keyphrases □ Mesoionic purinone analogs—synthesis and antibacterial properties of mesoionic thiazolo (or 1,3,4-thiadiazolo) [3,2-*a*]-pyrimidin-5,7-diones □ Mesoionic thiazolo (and 1,3,4-thiadiazolo) [3,2-*a*]pyrimidin-5,7-diones—synthesis and antibacterial screening □ Xanthine (mesoionic) analogs—synthesis and antibacterial properties of mesoionic thiazolo (and 1,3,4-thiadiazolo) [3,2-*a*]pyrimidin-5,7-diones □ Antibacterial activity—synthesis and screening of mesoionic thiazolo (or 1,3,4-thiadiazolo) [3,2-*a*]pyrimidin-5,7-diones

Formulation of a large, virtually unknown, class of mesoionic compounds, which are isoelectronic and isosteric to the purinones, was described previously (1, 2). They have been termed mesoionic purinone analogs because they cannot be satisfactorily represented by *any* covalent or single dipolar valence bond structure. Due to the large number of such analogs[1], they have been divided into two classes; Class I analogs are based upon known five-membered ring mesoionic systems (1, 3), and Class II analogs are based upon known six-membered ring mesoionic systems (2, 4). These two classes are further subdivided into xanthine, hypoxanthine, and purin-2-one analogs.

The compounds in this report, mesoionic thiazolo-[3,2-*a*]pyrimidin-5,7-diones[2] (I) and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5,7-diones[3] (II), are Class II mesoionic xanthine analogs. Quantum chemical studies of this subclass were reported (2), and convenient synthetic routes for their preparation were described (5). Molecular orbital studies of these analogs, in comparison with their covalent isomers, indicated an enhanced degree of chemical reactivity, an increase in charge separation and dipole moment, and an increase in electron affinity, which suggests an enhanced ability to participate in charge-transfer interactions as electron acceptors. Chemical studies (5) of the reaction of these compounds with amines suggested their potential function as selective acylating agents.



I



II

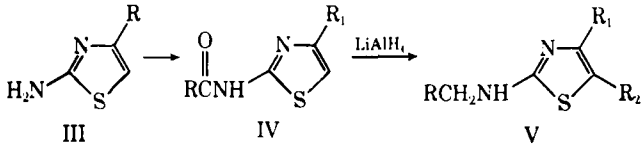
The synthesis of a number of derivatives of mesoionic thiazolo[3,2-*a*]pyrimidin-5,7-diones and 1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5,7-diones is reported here, along with the results of *in vitro* antibacterial screening as a part of an initial pharmacological investigation of this new class of compounds.

SYNTHESIS

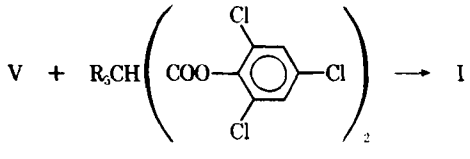
Compounds I*a*–I*h*, with the exception of I*e*, were prepared as outlined in Scheme I. 2-Aminothiazoles were acylated by the use of acyl chlorides or acid anhydrides to obtain the amides, IV (Table I), which were reduced *via* lithium aluminum hydride to the corresponding 2-alkylaminothiazoles, V (Table II). 2-Bromo-5-nitrothiazole was treated with 3,4-dichlorobenzylamine to obtain V*e*. Heating the 2-alkylaminothiazoles, V, with the malonate esters, VI (R_3 = H,CH_3), gave the mesoionic xanthine analogs, I, in good yield (Table III). The mesoionic thiadiazolopyrimidiones, II, were prepared by an analogous procedure.

EXPERIMENTAL[4]

***N*-(2-Thiazolyl)adamantane-1-carboxamide (IV*d*)**—A solution of adamantane-1-carboxylic acid chloride (6.0 g., 30 mmoles) in 30 ml. of tetrahydrofuran was added dropwise with stirring to a solution of 2-aminothiazole (3.0 g., 30 mmoles) and triethylamine (3.5 g., 35 mmoles) in 40 ml. of tetrahydrofuran at 0°. After the addi-



III IV V



VI

Scheme I

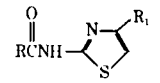
[1] Well over one hundred such analogs have been postulated (1, 2).

[2] Anhydro(8-substituted-5-hydroxythiazolo[3,2-*a*]pyrimidinium-7-one) hydroxide.

[3] Anhydro(8-substituted-5-hydroxy-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium-7-one) hydroxide.

[4] Proton magnetic resonance (PMR) spectra were obtained on a Varian T-60 spectrometer, and chemical shifts are reported relative to tetramethylsilane. IR spectra were obtained on a Beckman IR-18 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. All melting points were determined on a Mel-Temp melting-point apparatus and are uncorrected.

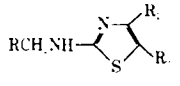
Table I—Properties of 2-Acylaminothiazoles, IV



Compound	R	R_1	Formula	Melting Point	Recrystallization Solvent[a]	Yield, %	Analysis, % Calc.	Found
IV*a*	Methyl	CH_3	$C_6H_8N_2OS$	133–134°[b]	T	92.6	—	—
IV*b*	3,4,5-Trimethoxyphenyl	H	$C_{13}H_{14}N_2O_4S$	173°	EA	79.4	C 53.03 H 4.80 Cl — N 9.52 S 10.87	53.06 4.69 — 9.36 11.08
IVc	3-Chlorophenyl	C_6H_5	$C_{16}H_{11}ClN_2OS$	146–148°	I	68.4	C 61.05 H 3.52 Cl 11.26 N 8.90 S 10.19	60.98 3.27 11.24 8.97 10.35
IV*d*	1-Adamantyl	H	$C_{14}H_{18}N_2OS$	198–199°	I	66.3	C 64.11 H 6.92 Cl — N 10.68 S 12.20	63.97 6.87 — 10.56 12.06

[a] T = tetrahydrofuran–petroleum ether, I = isopropanol, and EA = ethyl acetate. [b] Lit. (6) m.p. 134°.

Table II—Properties of 2-Alkylaminothiazoles, V



Compound	R	R_1	R_2	Formula	Melting Point	Recrystallization Solvent[a]	Yield, %	Analysis, % Calc.	Found
V*a*	Methyl	CH_3	H	$C_6H_{10}N_2S$	53°	H	75.0	C 50.67 H 7.08 Cl — N 19.69 S 22.55	50.62 7.21 — 19.54 22.61
V*b*	3,4,5-Trimethoxyphenyl	H	H	$C_{13}H_{16}N_2O_3S$	96–97°	T	57.2	C 55.69 H 5.75 Cl — N 9.99 S 11.44	55.48 5.68 — 10.04 11.40
V*c*	3-Chlorophenyl	C_6H_5	H	$C_{16}H_{13}ClN_2S$	93–94°	E	88.4	C 63.88 H 4.36 Cl — N 9.31 S 10.66	63.69 4.52 — 9.39 10.47
V*d*	1-Adamantyl	H	H	$C_{14}H_{20}N_2S$	104–105°	I	88.0	C 67.42 H 8.49 Cl — N 11.09 S 12.86	67.37 8.31 — 11.23 13.05
V*e*	3,4-Dichlorophenyl	H	NO_2	$C_{10}H_7Cl_2N_3O_2S$	150°	I	68.9	C 39.49 H 2.32 Cl 23.32 N 13.82 S 10.54	39.57 2.42 23.46 13.84 10.52

[a] H = hexane–petroleum ether, T = tetrahydrofuran–petroleum ether, E = ethanol 95%, and I = isopropanol.

tion was completed, stirring was continued at room temperature for 1 hr. Filtration and evaporation of the filtrate *in vacuo* gave the crude product. Recrystallization from isopropanol gave 5.2 g. (66.3%) of IV*d* as white crystals, m.p. 198–199°; IR (chloroform): 1675 (m) cm.$^{-1}$; NMR ($CDCl_3$): δ 1.9–2.2 (m, 15H, adamantyl protons), 7.2 (d, 1H, thiazole proton), and 7.7 (d, 1H, thiazole proton). Table I lists the analytical data.

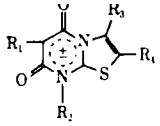
The 2-acylaminothiazoles, IV*a*–IVc (Table I), were prepared in the same manner as IV*d*.

2-(1-Adamantylmethylamino)thiazole (V*e*) A solution of IV*d* (1.3 g., 5 mmoles) in 20 ml. of tetrahydrofuran was added dropwise with stirring to a suspension of lithium aluminum hydride (0.2 g., 5.2 mmoles) in 20 ml. of tetrahydrofuran at 0°. After refluxing for 3 hr., water was slowly added at 0° until the evolution of hydrogen ceased. Filtration and evaporation of the filtrate *in vacuo* gave a colorless oil which crystallized upon standing. Recrystallization from isopropanol gave 1.1 g. (88%) of V*d* as white flakes, m.p. 104–105°; IR (chloroform): 3450 (m) and 2900 (s) cm.$^{-1}$; NMR ($CDCl_3$): δ 2.0 (m, 15H, adamantyl protons), 3.3 (s, 2H, —CH_2—), 6.2 (broad signal, 1H, —NH—), 6.8 (d, 1H), and 7.4 (d, 1H, thiazole protons). Table II lists the analytical data.

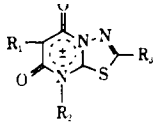
The 2-alkylaminothiazoles, V*a*–V*c* (Table II), were prepared in the same manner as V*d*.

2-(3,4-Dichlorobenzylamino)-5-nitrothiazole (V*e*)—2-Bromo-5-nitrothiazole (2.1 g., 10 mmoles) in 20 ml. of tetrahydrofuran was added dropwise with stirring to 3,4-dichlorobenzylamine (3.5 g., 20 mmoles) at 0°. After stirring at room temperature for 2 hr., the reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give a brown oil which crystallized upon standing overnight (16 hr.). Recrystallization from isopropanol gave 2.1 g. (68.9%) of V*e* as yellow crystals, m.p. 150°; IR (KBr): 1510 (s) and 1470 (s) cm.$^{-1}$; NMR (dimethyl sulfoxide-d_6): δ 4.6 (s, 2H, —CH_2—), 7.3–7.7 (m, 3H, phenyl protons), and 8.35 (s, 1H, thiazole proton). Table II lists the analytical data.

Table III—Properties of Mesoionic Thiazolo[3,2-*a*]pyrimidin-5,7-diones (I) and Mesoionic 1,3,4-Thiadiazolo[3,2-*a*]pyrimidin-5,7-diones (II)



I



II

Compound	R_1	R_2	R_3	R_4	Formula	Melting Point	Recrystallization Solvent[a]	Yield, %	Analysis, % Calc.	Found
I*a*	H	C_2H_5	CH_3	H	$C_9H_{10}N_2O_2S$	189–190°	I	81.6	C 51.41	51.45
									H 4.79	4.78
									N 13.33	13.49
									S 15.25	15.54
I*b*	CH_3	3,4,5-Trimethoxyphenyl	H	H	$C_{17}H_{18}N_2O_6S$	233–234°	M	97.8	C 56.33	56.21
									H 5.01	5.08
									N 7.73	7.67
									S 8.85	9.14
I*c*	CH_3	3-Chlorophenyl	C_6H_5	H	$C_{20}H_{15}ClN_2O_2S$	220–221°	A	52.2	C 62.74	63.06
									H 3.94	3.85
									N 7.32	7.28
									S 8.38	8.38
I*d*	CH_3	1-Adamantyl	H	H	$C_{18}H_{23}N_2O_2S$	210–211°	B	62.4	C 65.23	65.04
									H 7.00	7.10
									N 8.45	8.32
									S 9.67	9.80
I*e*	H	3,4-Dichlorophenyl	H	NO_2	$C_{13}H_7Cl_2N_3O_4S$	240° dec.	DA	32.7	C 42.95	43.30
									H 1.90	2.22
									N 11.29	10.89
									S 8.62	8.37
II*a*	CH_3	C_2H_5	H	—	$C_8H_9N_3O_2S$	214°	D	50.1	C 45.49	45.38
									H 4.30	4.48
									N 19.90	19.85
									S 15.18	15.32
II*b*	CH_3	C_6H_5	CH_3	—	$C_{14}H_{13}N_3O_2S$	227–228°	I	64.3	C 58.52	58.51
									H 4.56	4.61
									N 14.62	14.49
									S 11.16	11.27

[a] I = isopropanol, M = methanol, A = acetonitrile, B = benzene, DA = dimethylformamide–acetonitrile, and D = dimethylformamide.

Anhydro(6-methyl-7-hydroxy-8-(1-adamantylmethyl)thiazolo[3,2-*a*]pyrimidinium-5-one) Hydroxide (I*d*)—Compounds V*d* (0.37 g., 1.5 mmoles) and VI*b* (0.74 g., 1.5 mmoles) were mixed and heated on an oil bath (160°) until a clear melt resulted (5 min.). A slow stream of nitrogen was passed through the flask during the heating period to remove 2,4,6-trichlorophenol. When cool, the resultant product was triturated with 30 ml. of anhydrous ether and collected. Recrystallization from benzene gave 0.31 g. (62.4%) of I*d* as white crystals, m.p. 210–211°; IR (chloroform): 1685 (s) and 1630 (s) cm.$^{-1}$; NMR ($CDCl_3$): δ 2.3 (m, 18H, $-CH_3$ and adamantyl protons), 4.2 (s, 2H, $-CH_2-$), 7.7 (d, 1H), and 8.5 (d, 1H, thiazole protons). Table III lists the analytical data.

Anhydro(2,6-dimethyl-7-hydroxy-8-benzyl-1,3,4-thiadiazolo-[3,2-*a*]pyrimidinium-5-one) Hydroxide (II*b*)—2-Benzylamino-5-methyl 1,3,4-thiadiazole (7) (1.0 g., 5 mmoles) and VI*b* (2.4 g., 5 mmoles) were combined and heated at 160°, under a slow stream of nitrogen, until a clear melt resulted (3 min.). The product was triturated with 30 ml. of anhydrous ether, collected, and recrystallized from isopropanol to give 0.45 g. (64.3%) of II*b* as white crystals, m.p. 227–228°; IR (chloroform): 1685 (sh) and 1635 (s) cm.$^{-1}$; NMR ($CDCl_3$): δ 2.1 (s, 3H, $-CH_3$), 2.7 (s, 3H, thiazole $-CH_3$), 5.3 (s, 2H, $-CH_2-$), and 7.4 (s, 5H, phenyl protons). Table III lists the analytical data.

Compounds I*a*–I*c*, I*e*, and II*a* (Table III) were prepared in the same manner as I*d*, by heating at 160° until a clear melt resulted (about 1–5 min.) and then triturating with anhydrous ether to remove any unreacted starting material.

Partition Coefficients—The partition coefficient (*P*) of I*i* (R_1 = R_3 = R_4 = H, R_2 = C_2H_5) was determined in an octanol–aqueous buffer system (8). A master solution was prepared by dissolving I*i* in a pH 7.4 aqueous phosphate buffer to achieve a concentration of 2.9 $\times$ 10^{-4} mole/l. Dilutions of this solution were shaken for 10 min. with 20 ml. of octanol in Run A and with 25 ml. of octanol in Run B. These mixtures were then centrifuged, and the aqueous phase was examined by UV spectroscopy (242 nm.) to determine the aqueous concentration of I*i*. A blank was run without octanol to obtain the extinction coefficient in the buffer solution. The value of *P* was determined by dividing the concentration of I*i* in octanol by the concentration of I*i* in the buffer solution. Thus, the log *P* determined for I*i* was −1.41 for Run A and −1.44 for Run B. The log *P* for I*i* was taken as the average of the two runs or −1.425. Due to the additive nature of substituent constants (8, 9), the partition coefficients (log *P* values) could thus be calculated employing the π-values of the various substituents. Table IV lists the partition coefficients calculated for the mesoionic compounds employed in this investigation.

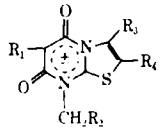
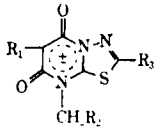
Antibacterial Testing Procedure—The following organisms were employed in this study: *Escherichia coli* (ATCC 11775), *Proteus vulgaris* (ATCC 13315), *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas aeruginosa* (ATCC 15691), *Bacillus subtilis* (ATCC 6051), *Streptococcus faecalis* (ATCC 8043), and *Staphylococcus aureus* (ATCC 12600). A lawn was prepared on Meuller-Hinton blood (5%) agar plates, using 1 ml. of a 24-hr. growth of the test organism in brain-heart infusion broth. Paper disks (6 mm.), im-

Table IV—Partition Coefficients Calculated for Mesoionic Thiazolo[3,2-*a*]pyrimidin-5,7-diones (I) and Mesoionic 1,3,4-Thiadiazolo[3,2-*a*]pyrimidin-5,7-diones (II)

Compound	Partition Coefficient (log *P*)
I*a*	−0.95
I*b*	+1.0
I*c*	+3.6
I*d*	+2.2
I*e*	+1.7
I*f*	+0.5
I*g*	+1.3
I*h*	−1.25
I*i*	−1.425[a]
II*a*	−2.3
II*b*	−0.2
II*c*	−2.8
II*d*	−2.3

[a] Determined experimentally.

Table V—Zones of Inhibition[a]

Compound	R_1	R_2	R_3	*E. coli*	*P. vulgaris*	*Ps. fluorescens*	*Ps. aeruginosa*	*B. subtilis*	*Strep. faecalis*	*Staph. aureus*
										
Ia	H	CH_3	CH_3	—[c]	7	—[c]	—[c]	10	—[c]	—[c]
Ib	CH_3	3,4,5-Trimethoxyphenyl	H	—[c]	11	14	—[c]	8	—[c]	10
Ic	CH_3	3-Chlorophenyl	C_6H_5	—[c]	—[c]	10	—[c]	—[c]	—[c]	7
Id	CH_3	1-Adamantyl	H	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
Ie[b]	H	3,4-Dichlorophenyl	H	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
If[d]	CH_3	C_6H_5	H	—[c]	18	16	8	16	—[c]	9
Ig[d]	$C_6H_5CH_2$	CH_3	H	—[c]	12	14	7	34	—[c]	—[c]
Ih[d]	H	$CH_2N(CH_3)_2$	H	—[c]	—[c]	10	9	27	9	8
										
IIa	CH_3	CH_3	H	—[c]	18	17	7	25	22	22
IIb	CH_3	C_6H_5	CH_3	—[c]	14	15	—[c]	—[c]	—[c]	—[c]
IIc[d]	H	CH_3	H	—[c]	32	12	7	30	13	19
IId[d]	H	CH_3	CH_3	—[c]	—[c]	—[c]	—[c]	29	—[c]	—[c]
Furazolidone				22	12	20	8	24	7	15
Sulfadimethoxine				20	10	16	—[c]	23	—[c]	—[c]
Sulfamerazine				20	—[c]	—	—[c]	27	—[c]	—[c]
Sulfamethoxypyridazine				26	13	—	—[c]	28	—[c]	—[c]

[a] Diameter (millimeters) of zones of inhibition. [b] $R_4 = NO_2$. [c] No inhibition. [d] Synthesis previously reported (5).

pregnated with 2 mg. of the test compounds, were placed on the agar and incubated for 24 hr. at 37°. *Ps. fluorescens* was incubated for 48 hr. at 25°.

Included on each plate were one or more disks[5] impregnated with the following commercially available antibacterial agents: furazolidone[6], 100 mcg./disk; sulfadimethoxine[7], 250 mcg./disk; sulfamerazine, 250 mcg./disk; and sulfamethoxypyridazine, 250 mcg./disk.

The tube dilution method was carried out using brain–heart infusion broth (5 ml.) with 10-, 25-, 50-, 100-, 250-, and 500-mcg./ml. concentrations of test compound. The broth was inoculated with 0.05 ml. of a 24-hr. growth of the test organism adjusted to a constant value of turbidity near that observed for full growth. Turbidity was measured at 6-hr. intervals using a spectrophotometer[8] (650 nm.). Full growth was usually reached in 12–18 hr. in a control tube.

RESULTS AND DISCUSSION

The zones of inhibition observed for Compounds *Ia*–*Ih* and *IIa*–*IId* against the test bacteria are shown in Table V. All but three of these mesoionic compounds (*Ie*, *Id*, and *IId*) exhibited bacteriostatic activity against two or more of the test organisms. None of the mesoionic compounds displayed any activity against *E. coli*. Against the other Gram-negative bacteria, Compounds *Ib*, *If*, *Ig*, *IIa*, and *IIc* showed zones of inhibition comparable to or greater than those of the standards. Compounds *Ih*, *IIa*, and *IIc* displayed significant zones of inhibition against Gram-positive organisms. Thiadiazolopyrimidiones *IIa* and *IIc* are notable for their activity against all of the test organisms except *E. coli*.

The activity of these compounds suggested the inclusion of a number of the monocyclic thiazole and thiadiazole precursors in the disk screening procedure, but no significant activity was observed.

To assay the levels of activity of the more active compounds, *If*, *IIa*, and *IIc* were tested by the broth tube dilution method, using nitrofurantoin[9] as a standard for comparison. The minimum inhibitory concentration (MIC) and the concentration inhibiting 50% of full growth (ID_{50}) for these compounds against *P. vulgaris* and *Staph. aureus* are shown in Table VI.

Compound *If* exhibited only a low level of activity against both organisms in this test. Compound *IIc* displayed greater activity than *If* in both cases, while *IIa* exhibited activity against *Staph. aureus* comparable to that of nitrofurantoin. Against *P. vulgaris*, *IIa* was about half as active as the standard. At a concentration of 250 mcg./ml., *IIa* was bactericidal against *P. vulgaris* but not against *Staph. aureus*. All three tested compounds showed some inhibition of growth at the 10-mcg./ml. level.

The range of lipophilicity represented by the derivatives *Ia*–*Ih* is quite large ($\Delta \log P = 4.85$). The most lipophilic mesoionic thiazolopyrimidiones, *Ic*, *Id*, and *Ie*, exhibited the lowest spectrum of activity. With the exception of *Ih*, the more active derivatives in this series have log *P* values between 0 and 1 (*If*, 0.5; *Ig*, 1.3; and *Ib*, 1.0). The thiadiazolopyrimidines *IIa*–*IId* are more hydrophilic and cover a more restricted range of lipophilicity (*IIb*, −0.2, to *IIc*, −2.8). Of more importance in this series is the apparent requirement for an unsubstituted 2-position (R_3 = H). Much more quantitative data concerning the activity of many more derivatives will be required before meaningful structure–activity relationships will be revealed.

The mesoionic thiazolopyrimidiones are nonbasic while the mesoionic thiadiazolopyrimidiones are very weakly basic. Both are stable to hydrolytic ring opening. Mesoionic thiazolopyrimidiones have been recrystallized from 5% aqueous hydrochloric acid (5). Refluxing either in aqueous solutions of strong mineral acids or alkali does produce ring-opened products (5). All reported derivatives have been found to be stable to heat and light in air.

Table VI—Antibacterial Activity[a] by the Broth Tube Dilution Method

Compound	*P. vulgaris* MIC	*P. vulgaris* ID_{50}	*Staph. aureus* MIC	*Staph. aureus* ID_{50}
If	500	500	500	500
IIa	100	40	50	15
IIc	200	90	225	125
Nitrofurantoin	50	22	40	18

[a] Micrograms per milliliter.

[5] Sensi-Discs, BBL, Cockeysville, MD 21030
[6] Furoxone.
[7] Madribon.
[8] Bausch & Lomb Spectronic 20.
[9] Furadantin.

CONCLUSION

The spectrum of *in vitro* activity exhibited by a significant number of the mesoionic thiazolo[3,2-*a*]pyrimidiones, I, and mesoionic thiadiazolo[3,2-*a*]pyrimidiones, II, suggests that these compounds should be considered a new and novel class of antibacterial agents. Several features of these compounds are significant. While they inhibited the growth of both Gram-negative and Gram-positive bacteria *in vitro*, no activity was displayed against *E. coli*. Furthermore, a number of the mesoionic compounds exhibited comparable or greater activities, as evidenced by their zones of inhibition, than several of the sulfa drugs or nitrofurans employed as standards.

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Pharmacokinetic Profile of Diazepam in Man following Single Intravenous and Oral and Chronic Oral Administrations

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Abstract □ Four subjects each received single intravenous and oral 10-mg. doses of diazepam and 10 mg. orally every 24 hr. for 15 days. The intravenous blood level data were fitted with a three-compartment open-model system containing both a "shallow" and a "deep" peripheral compartment. The "apparent" half-life of elimination of diazepam following intravenous administration ranged from 21 to 37 hr., and the calculated volume of distribution ranged from 160 to 205% of body weight. The rate at which diazepam returns to the central compartment from the deep peripheral compartment, k_{31}, was shown to be the rate-controlling factor in the elimination of diazepam and in the formation of desmethyldiazepam. Orally administered diazepam was rapidly and completely absorbed. Following chronic administration of 10 mg. diazepam every 24 hr., the minimum and maximum steady-state (plateau) levels of diazepam can be successfully predicted or calculated utilizing the pharmacokinetic parameters obtained following intravenous administration. Diazepam blood levels plateau at approximately Day 7 of treatment at twice the blood levels observed on Day 1. Desmethyldiazepam blood levels are within the range of the diazepam blood levels and exhibit an apparent half-life range from 50 to 99 hr. after the last dose of diazepam on Day 15.

Keyphrases □ Diazepam—pharmacokinetic profiles after single intravenous and oral and chronic oral administrations, man □ Pharmacokinetic profiles—diazepam after single intravenous and oral and chronic oral administrations, man □ Absorption kinetics—diazepam after single intravenous and oral and chronic oral administrations, man

Diazepam[1], 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one, is effective in the symptomatic relief of tension and anxiety states as well as for the relief of skeletal muscle spasms (1–8).

The drug exhibits a low aqueous solubility of 50 mcg./ml. and a pKa of 3.4 (9). The transfer characteristics of diazepam across the everted rat intestinal sac are consistent with good absorbability (10). This suggests that, once in solution, the permeability (absorbability) of diazepam across the GI mucosa will not be a rate-limiting factor following oral administration of the drug.

The major metabolic pathways of diazepam (Scheme I) have been described in various animal species and in man (11–17). In man, the major metabolite measurable in the bloodstream is desmethyldiazepam. In the urine, the glucuronide conjugate of oxazepam is the major detectable metabolite (11).

The present study was designed to elucidate the

[1] Valium, containing diazepam as its active ingredient (Hoffmann–La Roche Inc., Nutley, N. J.), was administered throughout the study.