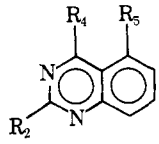
Table II. 5-Aryl-Substituted Quinazolines as Inhibitors of Dihydrofolate Reductase



No.	R_2	R_4	R_5	I_{50}, μM[a] Rat liver	I_{50}, μM[a] *S. faecium*
25	NH_2	NH_2	2-$C_{10}H_7S$–	0.12	0.056
26	NH_2	NH_2	3,4-$Cl_2C_6H_3S$–	0.16	0.29
27	NH_2	NH_2	2-$C_{10}H_7SO$–	45.0	44.0
28	NH_2	NH_2	3,4-$Cl_2C_6H_3SO$–	55.0	22.6
29	NH_2	NH_2	2-$C_{10}H_7SO_2$–	15.0	90.0
30	NH_2	NH_2	3,4-$Cl_2C_6H_3SO_2$–	57.0	1.4
31	NH_2	OH	2-$C_{10}H_7S$–	0.7	21.0
32	NH_2	OH	2-$C_{10}H_7SO_2$–	14.0	1.4
33	NH_2	NH_2	2-$C_{10}H_7SCH_2$–	0.010	0.028
34	NH_2	NH_2	4-$ClC_6H_4SCH_2$–	0.020	0.057
35	NH_2	NH_2	2-$C_{10}H_7(CH_2)_2$–	0.020	0.010
36	NH_2	NH_2	2-$C_{10}H_7CH$=CH– (cis)	0.30	0.037
37	NH_2	NH_2	2-$C_{10}H_7CH$=CH– (trans)	5.2	0.076
38	H	NH_2	3,4-$Cl_2C_6H_3S$–	>20	

[a] *Cf.* footnotes *a–c*, Table I.

polar groups in a hydrophobic region. As the distance between the 2,4-diaminopyrimidine moiety and the hydrophobic entity is increased, the inhibitory potency against both enzymes is enhanced (**33–35**), presumably by affording greater access to the hydrophobic bonding region. However, these are for the most part less effective than compounds **2–7** against the enzyme from either source. The planar trans olefin **37** is significantly less inhibitory toward the rat liver enzyme than its cis isomer **36** which in turn is less effective than the more flexible 2-naphthylethyl compound **35**. Conversely, each of these compounds is a moderately effective inhibitor of the bacterial enzyme suggesting that there are significant comformational differences between these isozymes. As expected, the exchange of the 4-NH_2 of **25** for 4-OH (**31**) caused a moderate loss in potency toward the mammalian enzyme and a much larger decrease with the bacterial enzyme. Conversely, modifications of the sulfone **29** in the same manner to yield **32** was ineffectual against the mammalian enzyme but caused an enhancement of inhibition with respect to the bacterial enzyme.

Acknowledgments. The authors wish to express appreciation to Mr. F. C. Walker, III, for performing the rat liver enzyme assays and to Dr. E. A. Steck of the Walter Reed Army Institute of Research for providing several key samples employed in this research.

References

(1) W. T. Ashton, F. C. Walker, III, and J. B. Hynes, *J. Med. Chem.*, **16**, 694 (1973) (paper 1).
(2) J. Davoll and A. M. Johnson, *J. Chem. Soc. C*, 997 (1970).
(3) A. M. Albrecht and D. J. Hutchison, *Mol. Pharmacol.*, **6**, 323 (1970).
(4) E. Fölsch, G. Abboud, E. Gralla, and J. R. Bertino, *Ann. N. Y. Acad. Sci.*, **186**, 501 (1971).
(5) D. J. Hutchison, *Ann. N. Y. Acad. Sci.*, **186**, 496 (1971).
(6) J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 812 (1972).
(7) P. E. Thompson, A. Bayles, and B. Olszewski, *Exp. Parasitol.*, **25**, 32 (1969).
(8) P. E. Thompson, A. Bayles, and B. Olszewski, *Amer. J. Trop. Med. Hyg.*, **19**, 12 (1970).
(9) E. F. Elslager, O. D. Bird, J. Clarke, S. C. Perricone, D. F. Worth, and J. Davoll, *J. Med. Chem.*, **15**, 1138 (1972).
(10) E. F. Elslager and J. Davoll, "Lectures in Heterocyclic Chemistry," Vol. II, R. N. Castle and L. B. Townsend, Ed., HeteroCorporation, Orem, Utah, 1974, pp S-97–S-133.
(11) J. B. Hynes, W. T. Ashton, H. G. Merriman, III, and F. C. Walker, III, *J. Med. Chem.*, **17**, 682 (1974).
(12) J. H. Freisheim, C. C. Smith, and P. M. Guzy, *Arch. Biochem. Biophys.*, **148**, 1 (1972).
(13) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, Chapter 10, and references cited therein.
(14) W. T. Ashton and J. B. Hynes, *J. Med. Chem.*, **16**, 1233 (1973).

Mesoionic Purinone Analogs. 7. *In Vitro* Antibacterial Activity of Mesoionic 1,3,4-Thiadiazolo[3,2-*a*]pyrimidine-5,7-diones†

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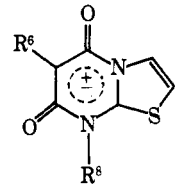
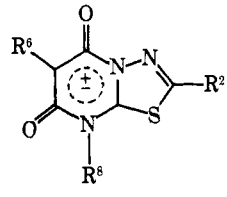
The discovery of *in vitro* antibacterial activity of mesoionic thiazolo[3,2-*a*]pyrimidine-5,7-diones (**1**) and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones (**2**) has been recently reported.[1,‡] These compounds are members of a large, virtually unknown, class of mesoionic structures, termed mesoionic purinone analogs, which are isoelectronic and isosteric to the purinones: xanthine, hypoxanthine, or purin-2-one. The formulation, classification, and quantum chemical study of a large number of these heterocyclic structures have been described.[2,3] In this report, a series of alkyl- and aryl-substituted mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones (**2**), mesoionic xanthine analogs, was prepared and examined for antibacterial activity in order to develop structure–activity relationships leading to more active derivatives.



1



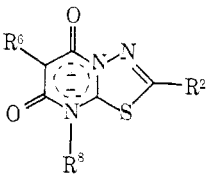
2

Chemistry. Compounds **2a–t**, Table I, were prepared by the condensation of 2-*sec*-amino-1,3,4-thiadiazoles with malonate esters as previously described.[1,4] The required 2-alkylamino- (or 2-arylamino-) thiadiazoles, unsubstituted in the 5 position, were prepared from the corresponding alkylamines by conversion to alkyl isothiocyanates (Kaluza reaction[5]) which were then treated with hydrazine to give 4-alkyl thiosemicarbazides (Scheme I). Treatment of 4-substituted thiosemicarbazides with triethyl orthoformate, under acid catalysis, gave the desired thiadiazoles **4** in high yield.[6]

†Taken in part from the dissertation submitted by R. A. Glennon to SUNY/Buffalo in partial fulfillment of the requirements for the Ph.D. degree. Presented in part at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973, MEDI 71.

‡ Alternative nomenclature: anhydro-8-alkyl-5-hydroxy-7-oxothiazolo[3,2-*a*]pyrimidinium hydroxide and anhydro-8-alkyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium hydroxide.

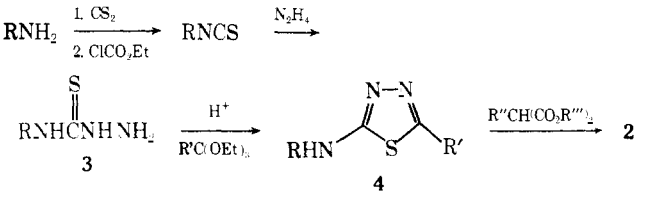
Table I. Mesoionic 1,3,4-Thiadiazolo[3,2-*a*]pyrimidine-5,7-diones



Compd	R^2	R^6	R^8	Yield, %[a]	Mp, °C	Crystn solvent[b]	Formula[c]	MIC, μg/ml *Staph. aureus*	MIC, μg/ml *P. vulgaris*
2a	H	H	Me	78	223–224	AA	$C_6H_5N_3SO_2$	500	250
2b	H	Me	Me	14	215–216	D	$C_7H_7N_3SO_2$	500	250
2c	Me	Me	Me	28	302–303	D	$C_8H_9N_3SO_2$	>1000	>1000
2d[d]	H	H	Et	73	208–209	A	$C_7H_7N_3SO_2$	250	500
2e[d]	H	Me	Et	50	214–215	D	$C_8H_9N_3SO_2$	125	125
2f[d]	Me	H	Et	36	208–210	I	$C_8H_9N_3SO_2$	>1000	>1000
2g	H	Et	Et	24	181–182	I	$C_9H_{11}N_3SO_2$	250	125
2h	H	$PhCH_2$	Et	82	235–236	D	$C_{14}H_{13}N_3SO_2$	500	250
2i	H	H	Pr	62	190–191	D	$C_8H_9N_3SO_2$	125	125
2j	H	Me	Pr	53	208–209	D	$C_9H_{11}N_3SO_2$	125	125
2k	H	Me	*i*-Pr	53	210–211	D	$C_9H_{11}N_3SO_2$	500	250
2l	H	Me	Bu	44	205–206	D	$C_{10}H_{13}N_3SO_2$	125	65
2m	H	Me	*i*-Bu	63	204–205	D	$C_{10}H_{13}N_3SO_2$	125	65
2n	H	Me	Pentyl	87	190–191	A	$C_{11}H_{15}N_3SO_2$	125	65
2o	H	Me	Hexyl	18	185–187	A	$C_{12}H_{17}N_3SO_2$	125	125
2p	H	Me	Heptyl	29	186–187	A	$C_{13}H_{19}N_3SO_2$	250	250
2q	H	H	$PhCH_2$	39	205–206	D	$C_{12}H_9N_3SO_2$	500	500
2r	H	Me	$PhCH_2$	71	213–214	DA	$C_{13}H_{11}N_3SO_2$	250	250
2s[d]	Me	Me	$PhCH_2$	64	227–228	I	$C_{14}H_{13}N_3SO_2$	>1000	>1000
2t	H	Me	Ph	66	225–226	D	$C_{12}H_9N_3SO_2$	250	250
Nitrofurantoin								125	65

[a]Condensation step only. [b]AA, $MeCO_2H$; A, MeCN; D, DMF; DA, DMA; I, *i*-PrOH. [c]All compounds were analyzed for C, H, N, and S. [d]Reference 1.

Scheme I



5-Substituted 2-alkylaminothiadiazoles are conveniently prepared from the corresponding 5-substituted 2-aminothiadiazoles by acylation and $LiAlH_4$ reduction of the resulting amides. 5-Methyl-2-methylamino-1,3,4-thiadiazole was prepared by the acid-catalyzed condensation of 4-methyl thiosemicarbazide with trimethyl orthoacetate in methanol.[6]

Biological Activity. The minimum inhibitory concentrations (MIC) of these compounds and nitrofurantoin, determined by a tube dilution method[1] employing Trypticase-Soy broth (BBL), against *Staphylococcus aureus* (ATCC 12600) and *Proteus vulgaris* (ATCC 13315) are shown in Table I. It is evident that a methyl group at C-2 (**2c**, **2f**, and **2s**) results in a complete loss of activity. For this reason compounds with substituents at the 2 position were not further investigated. There appears to be a slight advantage favoring a methyl group at C-6 (**2d** *vs.* **2e** and **2q** *vs.* **2r**) while ethyl or benzyl groups at this position are tolerated. Increasing the length of an alkyl chain at the 8 position increases the activity against both organisms, with optimal activity being obtained with butyl and pentyl groups. Benzyl, phenyl, or longer alkyl chains at this position resulted in slightly lower activities. Attempts to correlate activity with partition coefficients were not undertaken due to the limited range of observed activities.

The antibacterial activities of compounds **2j**, **2l**, **2n**, and **2t** were determined against *Escherichia coli*, *Streptococcus facaelis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Significant activity (MIC <250 μg/ml) was found against only *B. subtilis* with MIC's of 30, 60, and 60 μg/ml for **2j**, **2l**, and **2n**, respectively. Compound **2t** exhibited a MIC of 250 μg/ml against *Candida albicans*. Compounds **2i**, **2l**, **2n**, and **2p** exhibited *in vitro* activity at 100 μg/ml against *Schistosoma mansoni* while the latter two compounds also exhibited *in vitro* activity at the same level against *Trypanosoma cruzi*. None of these four compounds displayed antiviral activity when tested against parainfluenza, rhinovirus, and adeno and herpes type I viruses. Antimalarial assays[7] against *Plasmodium berghei* in mice revealed no significant activity for compounds **2d**, **2e**, **2i**, **2l**, **2q**, and **2t**.

The acute interperitoneal toxicities in mice for compounds **2j** and **2l**, administered in aqueous methylcellulose suspensions, were found to be 60 and 175 mg/kg, respectively.

Compound **2e** was found to be stable in aqueous solution at 37° for >4 days as evidenced by integration of the characteristic H-2 pmr signal (δ 9.5). Mesoionic thiazolo[3,2-*a*]pyrimidines **1**[4] and mesoionic thiazolo- or 1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine-5,7-diones[8] react with amines by apparent nucleophilic attack at the 5 position and cleavage of the N_4–C_5 bond resulting in a ring-opened amide product. Similarly, **2e** reacts rapidly with primary alkylamines ($t_{1/2}$ *ca.* 5 min with equimolar benzylamine in MeOH at 37°). This reaction is *ca.* 1000 times faster than the corresponding reaction of **1** (R^6 = Me; R^8 = Et) under identical conditions.§ This reactivity offers a possible hypothesis for the low to moderate levels of antimicro-

§The antibacterial activities of alkyl derivatives of **1**, though not as extensively studied as for **2**, are lower than the corresponding derivatives of **2**.[1] Mesoionic thiadiazolo[3,2-*a*]-*s*-triazine-5,7-diones are not stable in aqueous solution and did not exhibit antibacterial activity.

bial activities of these compounds involving acylation by the mesoionic compound at unknown loci.

1-Methyl-3-isobutylxanthine was the most active of a series of 57 xanthine derivatives studied for their lipolytic potencies in epididymal fat cells.[9] Close correlation between this lipolytic activity and inhibition of cyclic AMP phosphodiesterase (PDE) was observed. Detracting somewhat in this case from this parallel structural relationship is the increase in PDE inhibition produced by alkyl groups in the 8 position of theophylline.[10] Compounds in this report are currently being evaluated as potential inhibitors of c-AMP phosphodiesterase.

Experimental Section

Melting points (uncorrected) were determined on a Mel-Temp melting point apparatus. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

n-**Pentyl Isothiocyanate.** Pentylamine (17.4 g, 0.2 mol) was added dropwise with stirring to a mixture of CS_2 (15.2 g, 0.2 mol) and 8% aqueous sodium hydroxide (100 ml). The mixture was stirred at room temperature for 1 hr, heated on a steam bath for 1 hr, and then chilled in an ice bath. Ethyl chloroformate (21.6 g, 0.2 mol) was added dropwise to the chilled mixture which was then stirred for 1 hr at room temperature. The organic layer was separated and distilled to give 18 g (64.7%) of *n*-pentyl isothiocyanate, bp 54–56° (3 mm) (lit.[11] bp 191°).

The Pr, *i*-Pr, Bu, *i*-Bu, hexyl, heptyl, and benzyl isothiocyanates were prepared in the same manner and used without further purification.

4-*n*-**Pentylthiosemicarbazide** (3, **R** = *n*-C_5H_{11}). Hydrazine (3.2 g, 0.1 mol) was *slowly* added to a solution of pentyl isothiocyanate (12.9 g, 0.1 mol) in Et_2O (50 ml) at 0°.‡ The reaction mixture was stirred at room temperature for 1 hr and the product collected by filtration. Recrystallization from PhH–petroleum ether gave 14.8 g (92%) of 3 (R = *n*-C_5H_{11}) as white crystals, mp 46–48°. *Anal.* ($C_6H_{15}N_3S$) C, H, N, S.

4-*n*-**Hexylthiosemicarbazide** (3, **R** = *n*-C_6H_{13}) was prepared by the above method from *n*-hexyl isothiocyanate and obtained in 87% yield following recrystallization from PhH/petroleum ether, mp 50–51°. *Anal.* ($C_7H_{17}N_3S$) C, H, N, S.

4-*n*-**Heptylthiosemicarbazide** (3, **R** = *n*-C_7H_{15}) was prepared by the above method from *n*-heptyl isothiocyanate and obtained in 93% yield following recyrstallization from petroleum ether, mp 49–50°. *Anal.* ($C_8H_{19}N_3S$) C, H, N, S.

2-*n*-**Pentylamino-1,3,4-thiadiazole** (4, **R** = *n*-C_5H_{11}; **R′ = H**). A solution of 3 (R = *n*-C_5H_{11}) (3.2 g, 20 mmol), triethyl orthoformate (3.0 g, 20 mmol), and concentrated HCl (0.1 ml) in 95% EtOH (20 ml) was stirred at room temperature for 1 hr and then refluxed for 1 hr. The solvent was removed *in vacuo* and the residual oil was dissolved in EtOAc (10 ml) and petroleum ether added to give a white cyrstalline precipitate. Recrystallization from EtOAc–petroleum ether gave 3.0 g (88%) of 4 (R = *n*-C_5H_{11}; R′ = H), mp 49–50°. *Anal.* ($C_7H_{13}N_3S$) C, H, N, S.

2-*n*-**Hexylamino-1,3,4-thiadiazole** (4, **R** = *n*-C_6H_{13}; **R′ = H**) was prepared by the above method from 3 (R = *n*-C_6H_{13}) and obtained in 61% yield following recrystallization from PhH–petroleum ether, mp 80–81°. *Anal.* ($C_8H_{15}N_3S$) C, H, N, S.

2-*n*-**Heptylamino-1,3,4-thiadiazole** (4, **R** = *n*-C_7H_{15}, **R′ = H**) was prepared by the above method from 3 (R = *n*-C_7H_{15}) and obtained in 87% yield following recrystallization from PhH–petroleum ether, mp 70–71°. *Anal.* ($C_9H_{17}N_3S$) C, H, N, S.

2-**Isopropylamino-1,3,4-thiadiazole** (4, R = *i*-C_3H_7; **R′ = H**) was prepared by the above method from 3 (R = *i*-C_3H_7) and obtained in 89% yield following recrystallization from THF–petroleum ether, mp 109–110°. *Anal.* ($C_5H_9N_3S$) C, H, N, S.

2-Ethylamino-5-methyl-1,3,4-thiadiazole (4, **R = C_2H_5; R′ = CH_3**). 2-Acetamido-5-methyl-1,3,4-thiadiazole (1.5 g, 10 mmol) was added in small portions to a stirred suspension of $LiAlH_4$ (0.38 g, 10 mmol) in THF (25 ml) at 0°. After refluxing for 2 hr, water was added dropwise until the evolution of gas ceased. The resultant mixture was filtered and the filtrate evaporated *in vacuo*. Recrystallization of the residue from 95% EtOH gave 0.72 g (53%) of 4 (R = C_2H_5; R′ = CH_3), mp 67–68° (lit.[12] mp 67–68°).

2-Benzylamino-5-methyl-1,3,4-thiadiazole (4, **R = $PhCH_2$; R′ = CH_3**) was prepared by the above method from 2-benzamido-5-methyl-1,3,4-thiadiazole and obtained in 56% yield following recrystallization from EtOH, mp 134–136° (lit.[13] mp 138–139°).

Anhydro-6-methyl-8-*n*-pentyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium Hydroxide (2n). An intimate mixture of 4 (R = *n*-C_5H_{11}; R′ = H) (0.86 g, 5 mmol) and bis(2,4,6-trichlorophenyl) methylmalonate (2.38 g, 5 mmol) was heated on an oil bath (160°) under a slow stream of N_2 until a clear melt was obtained (4 min). The cooled oil was triturated with Et_2O and the resulting precipitate collected by filtration. Recrystallization from MeCN gave 1.1 g (87%) of **2n** as white crystals, mp 190–191°. *Anal.* ($C_{11}H_{15}N_3SO_2$) C, H, N, S.

The mesoionic thiadiazolopyrimidines **2a–t** (Table I) were prepared in an identical manner with that for **2n** employing either methyl, ethyl, benzyl, or the unsubstituted bis(2,4,6-trichlorophenyl) malonate ester.[14]

Acknowledgment. This work was supported in part by the U. S. Army Medical Research and Development Command under Contract DADA-17-73-C-3054. This is Contribution No. 1219 from the Army Research Program on Malaria. We are indebted to Dr. Roland K. Robins and the ICN Nucleic Acid Research Institute for data concerning antiviral and *in vitro* antiparasitological assays.

References

(1) R. A. Coburn and R. A. Glennon, *J. Pharm. Sci.*, **62**, 1785 (1973).
(2) R. A. Coburn, *J. Heterocycl. Chem.*, **8**, 881 (1971).
(3) R. A. Coburn, R. A. Carapellotti, and R. A. Glennon, *J. Heterocycl. Chem.*, **10**, 479 (1973).
(4) R. A. Coburn and R. A. Glennon, *J. Heterocycl. Chem.*, **10**, 486 (1973).
(5) M. L. Moore and F. C. Crossley, *Org. Syn.*, **21**, 81 (1941); J. E. Hodgkins, W. P. Rieves, and Y. G. Liu, *J. Amer. Chem. Soc.*, **83**, 2532 (1961).
(6) R. A. Coburn, B. Bhooshan, and R. A. Glennon, *J. Org. Chem.*, **38**, 3947 (1973).
(7) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(8) R. A. Coburn and B. Bhooshan, *J. Org. Chem.*, **38**, 3868 (1973).
(9) J. A. Beavo, N. L. Rogers, O. B. Crofford, J. G. Hardman, E. W. Sutherland, and E. V. Newman, *Mol. Pharmacol.*, **6**, 597 (1970).
(10) E. B. Goodsell, H. H. Stein, and K. J. Wenzke, *J. Med. Chem.*, **14**, 1202 (1971).
(11) G. M. Dyson and R. F. Hunter, *Recl. Trav. Chim. Pays-Bas*, **45**, 421 (1926).
(12) C. W. Whitehead and J. J. Trauciso, *J. Amer. Chem. Soc.*, **77**, 5872 (1955).
(13) H. Saikachi and M. Kanaoka, *Yakugaka Zasshi*, **81**, 1333 (1961); *Chem. Abstr.*, **56**, 7304 (1962).
(14) T. Kappe and W. Lube, *Monatsh. Chem.*, **103**, 781 (1971).

‡On one occasion a moderate rate of addition of hydrazine to hexyl isothiocyanate resulted in a violent eruption of the reaction mixture.

Synthesis of the α and β Anomers of 1-(2-Deoxy-D-ribofuranosyl)-4-pyridone†

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Deaza and deoxy analogs of the naturally occurring nucleosides recently have been popular design factors in the search for new cancer chemotherapeutic agents. While the 1-deaza analogs of uridine and 2′-deoxyuridine[1] were unstable and were not growth inhibitory, the 3-deaza analogs clearly show a broad spectrum of antiviral activity.[2]

Previous studies of the inhibition of thymidylate syn-

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