um bicarbonate and water and were dried (Na_2SO_4). Evaporation afforded 7.23 g of an oil which was dissolved in 18 ml of dry ethanol and added dropwise to 18 ml of ethanolic hydrogen bromide. The solution was diluted with benzene and evaporated and the residue was crystallized from ethanol–ether to afford 6.74 g (56%) of **10**, mp 144°. *Anal.* ($C_{19}H_{20}BrNO_3$) C, H, N.

2-Benzyl-2,3-dihydro-4(1*H*)-isoquinolone (11). The keto ester **10** (6.80 g, 0.022 mol) (as the free base) was dissolved in 40 g of 60% sulfuric acid. The resulting solution was stirred under reflux for 4 hr, cooled to room temperature, diluted with 200 ml of water, and cooled in an ice bath and the pH was adjusted to 9 by dropwise addition of 20% sodium hydroxide. The basic mixture was extracted with three 150-ml portions of ether which were combined, washed with water, and dried ($MgSO_4$). Evaporation afforded 3.70 g (68%) of **11** as a golden oil: ir (liquid film) 1680 cm^{-1}; nmr 8.05 (m, 1 H, aromatic), 7.40 (m, 8 H aromatic), 3.73 and 3.78 (2 H each, s, CH_2NCH_2), 3.40 (s, C-3 methylene).

2-Benzyl-2,3-dihydro-4(1*H*)-isoquinolone Oxime (12). The ketone **11** (3.70 g, 0.015 mol) was dissolved in 70 ml of ethanol and added to a solution of hydroxylamine hydrochloride (8.89 g) in 50 ml of water and 32 ml of 10% sodium hydroxide. The resulting solution was refluxed on a steam bath for 1 hr, filtered while hot, and allowed to stand overnight at room temperature. The oxime **12** (1.80 g, 47%) was collected by filtration and recrystallized from cyclohexane: mp 161°. *Anal.* ($C_{16}H_{16}N_2O$) C, H, N.

2-Benzyl-2,3-dihydro-4(1*H*)-isoquinolone Oxime Acetate (13). The oxime **12** (7.52 g, 0.03 mol) was dissolved in 47 ml of anhydrous pyridine along with 68 ml of acetic anhydride. The solution was heated on a steam bath for 20 min, cooled to room temperature, poured onto ice, and made basic (pH 8) by dropwise addition of 10% sodium carbonate. The cold, basic mixture was extracted immediately with ether and the ether solution was washed with water and dried ($MgSO_4$). Thorough evaporation *in vacuo* afforded **13** (8.30 g, 93%) as a yellow solid: mp 97–99°; nmr 8.11 (m, 1 H, aromatic), 7.16 (m, 8 H, aromatic), 3.70 and 3.80 (2 H each, s, CH_2NCH_2), 3.63 (s, C-3 methylene), 2.18 (s, CH_3).

2-Benzyl-1,2,3,4-tetrahydro-4-dimethylaminoisoquinoline (2). To a cold stirring solution of the oxime acetate **13** (6.35 g, 0.215 mol) in 110 ml of tetrahydrofuran was added dropwise 121 ml of a 1 *M* solution of borane in tetrahydrofuran. After the addition was complete, the mixture was stirred 70 hr at room temperature. The mixture was again cooled in an ice–salt bath and 84 ml of 5% hydrochloric acid was cautiously added. The solvent was evaporated and the aqueous residue was washed with ether and made basic (pH 12) by the addition of 10 *N* potassium hydroxide. Solid sodium chloride was added and the basic mixture was extracted with several portions of ether which were combined, washed with saturated sodium chloride, and dried ($MgSO_4$). Evaporation afforded 3.45 g (67%) of **14** as a brown viscous oil. The nmr spectrum of the crude amine gave the expected integration and exhibited a two proton singlet which disappeared upon treatment of the solution with deuterium oxide.

The crude amine **14** (3.45 g, 0.0145 mol) was dissolved in 41 ml of acetonitrile to which was added 5.7 ml of 37% formaldehyde and 1.35 g of sodium cyanoborohydride. After 15 min of stirring, sufficient glacial acetic acid was added to adjust the pH to 7. Stirring was continued for 45 min with glacial acetic acid being added as required to maintain a pH of 7. The acetonitrile was evaporated *in vacuo* and 50 ml of 2 *N* potassium hydroxide was added to the residue. The basic mixture was extracted with several portions of ether which were combined, washed with 0.5 *N* potassium hydroxide, and extracted with 10% hydrochloric acid. The acid extracts were made basic (pH 10) with solid potassium hydroxide and were extracted with ether. The ether extracts were combined, dried ($MgSO_4$), and evaporated to afford 3.05 g of crude **2** as a brown oil. The crude product was chromatographed on a column of 150 g of basic aluminum oxide (activity grade I). Elution with petroleum ether (bp 60–70°) followed by benzene–petroleum ether and benzene–ethyl acetate afforded **2** (2.20 g, 57%) as a tan oil. A sample of **2** was rechromatographed as described above for pharmacological testing and elemental analysis: nmr 6.70–7.66 (m, 9 H, aromatic), 3.88 (m, C-4 H), 3.63 and 3.51 (2 H each, s, CH_2NCH_2), 2.75 (m, C-3 methylene), 2.30 (s, NC_2H_6); mass spectrum *m/e* (rel intensity) 221 (66), 220 (38), 146 (100), 132 (62), 91 (22). *Anal.* ($C_{18}H_{22}N_2$) C, H, N.

Pharmacology. Testing was carried out on the isolated guinea pig ileum which was prepared according to a standard method.[10] The ileum was bathed in Tyrodes solution at 37° and bubbled with air. Tissues were allowed to stabilize for a minimum of 15 min before introduction of agonists. Histamine was then added at 3-min intervals until reproducible contractions were obtained. Antagonists were dissolved in 0.1 *N* hydrochloric acid and an aliquot was dissolved in Tyrodes solution for pharmacological evaluation. Antagonists were allowed to remain in contact with the ileal tissue for 15 min prior to the addition of 4×10^{-6} *M* histamine or 4×10^{-7} *M* acetylcholine. The ED_{50} values cited in the discussion are the results of three determinations at three dose levels. The values in parentheses are standard errors.

Acknowledgments. P. E. H. gratefully acknowledges the assistance of Siv R. Goulding and Ahmed Abou El Sood. This work was supported by Grant GM 15477 from the National Institutes of Health, U. S. Public Health Service.

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Quinazolines as Inhibitors of Dihydrofolate Reductase. 2[1,†]

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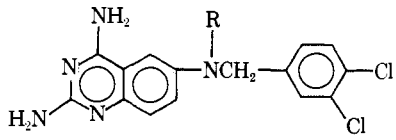
Received April 12, 1974

During the past decade a wide variety of quinazoline derivatives has been synthesized and evaluated as potential chemotherapeutic agents. Noteworthy examples of classical quinazoline antifolates are chlorasquin and methasquin which are highly potent inhibitors of dihydrofolate reductase. These compounds have also been studied extensively as potential antineoplastic agents.[2-5] On the other hand, the discovery of the potent antiprotozoan activity of 2,4-diamino-6-(benzylamino)quinazolines, **1a–c**, has stimulated the development of a wide variety of potent nonclassical quinazoline antifolates.[6-9] These efforts have culminated in the selection of two 2,4-diamino-6-

† A portion of this research was supported by NIH General Research Support R.R. 05420 to the Medical University of South Carolina (J. B. H.), while the remainder was performed under NIH Grant CA-11666 (J. H. F.). A preliminary account of this work was presented at the 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1–5, 1974, Abstracts, MEDI-56.

‡ Research Career Development Award (KO4-CA-70449) from the National Cancer Institute.



1a, R = H
b, R = NO
c, R = CH_3

(arylthio)quinazolines, 4 and 5 (*cf.* Table I), for clinical evaluation as antimalarial agents.[10]

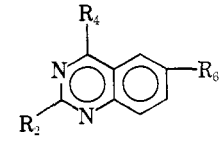
As an adjunct to a synthetic program concerning modifications of 4 and **5**, we initiated a systematic study of the inhibition of rat liver dihydrofolate reductase by quinazolines bearing relatively simple substituents.[1] It was found that among the 2,4-diaminoquinazolines studied, significant activity enhancement could be obtained by the introduction of a small hydrophobic group in the 5 position and to a lesser degree in the 6 position. The most potent modifications contained nonpolar substituents in both positions. The current study involves an extension of this work to include compounds containing more complex substitution with particular emphasis being placed upon those having potential clinical utility. In addition to providing fundamental information concerning structural requirements necessary for effective inhibition of dihydrofolate reductase by nonclassical quinazolines, it was believed that this information might prove to be of value in anticipating potential host toxicity. The enzymes employed in this study were the dihydrofolate reductases obtained from rat liver as well as from *Streptococcus faecium*, the latter source being included in order to determine the extent to which isozyme specificity is displayed by compounds of this class.

The results obtained with 23 6-arylthioquinazolines and derivatives are presented in Table I. The 2,4-diaminoquinazolines **2–7** were initially synthesized by Elslager and coworkers,[10] while the preparation of the remaining analogs was described in a recent communication.[11] Compounds **2–7** were potent inhibitors of the mammalian enzyme with **4** being the most potent and **5** nearly an order of magnitude less effective. Therefore, assuming equal antimalarial efficacy, **5** may possess greater clinical utility than **4** by virtue of reduced folate deficiency toxicity in man. Since the bacterial enzyme assay was performed using greater than three times the concentration of the substrate dihydrofolate, each of the 2,4-diamino derivatives is more inhibitory toward the bacterial enzyme with **5** displaying the most dramatic increase. It should be noted that **5** and **7** compare favorably with methotrexate as inhibitors of the *S. faecium* enzyme when the latter is assayed under these conditions.[12]

Replacement of the 4-NH_2 group by a 4-OH (**8–12**) results in only a three- to tenfold decrease in activity against the rat liver enzyme, while more substantial losses in potency are observed with respect to the bacterial enzyme. In the case of pyrimidines and pteridines, the 2-amino-4-hydroxy derivatives are normally two to three orders of magnitude poorer inhibitors of mammalian reductase than their 2,4-diamino counterparts.[13] The 2-amino-4-mercapto modifications (**13** and **14**) are still less effective than their 4-OH counterparts with respect to either enzyme. Furthermore, the sulfinyl or sulfonyl bridge is particularly detrimental to activity of 4-OH or 4-SH derivatives against the *S. faecium* enzyme (**10–12, 14**). Conversely, in the case of the 2,4-diamino configuration, **4** is comparable in inhibitory potency against either enzyme, while **3** and **7** are significantly more effective against that from the bacterial source. A conformational difference in binding between the 2,4-diaminoquinazolines on one hand and their 4-OH or 4-SH analogs on the other is thus implied. All modifications involving substituents at the 2 position of the quinazoline ring (**15–24**) led to drastic reductions in inhibitory potency in particular with respect to the bacterial enzyme. Therefore, the presence of a 2-NH_2 appears to be a necessary requirement for effective inhibition of either enzyme. Acetylation of the 2-NH_2 group of **11** to yield **24** results in a 71-fold loss in inhibitory potency with regard to the rat liver enzyme and an 1110-fold reduction with respect to the bacterial enzyme. This effect may be attributed to a reduction in the basicity of the heterocyclic ring and/or to a lack of bulk tolerance by the enzymes.

A series of 5-arylethyl-, 5-arylthio-, and 5-arylthiomethyl-2,4-diaminoquinazolines[14] was also included in this study and the results obtained are summarized in Table II. The 5-arylthio-2,4-diaminoquinazolines (**25** and **26**) are moderately inhibitory toward each enzyme but are substantially less active than their isomers **2** and **6**. However, oxidation to the sulfinyl (**27** and **28**) or sulfonyl (**29** and **30**) analogs caused a marked decrease in activity. This is in contradistinction to results obtained with the 6-position isomers and may be due to a lack of tolerance for highly

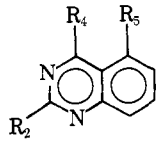
Table I. 6-Arylthioquinazolines as Inhibitors of Dihydrofolate Reductase



No.	R_2	R_4	R_6	I_{50}, μM[a] Rat liver[b]	I_{50}, μM[a] *S. faecium*[c]
2	NH_2	NH_2	2-$C_{10}H_7S$–[d]	0.007	0.007
3	NH_2	NH_2	2-$C_{10}H_7SO$–[d]	0.007	0.002
4	NH_2	NH_2	2-$C_{10}H_7SO_2$–[d]	0.004	0.004
5	NH_2	NH_2	3-$(CF_3)C_6H_4S$–[d]	0.036	0.0007
6	NH_2	NH_2	3,4-$Cl_2C_6H_3S$–[d]	0.011	0.0057
7	NH_2	NH_2	3,4-$Cl_2C_6H_3SO_2$–[d]	0.009	0.0007
8	NH_2	OH	2-$C_{10}H_7S$–	0.045	0.18
9	NH_2	OH	3,4-$Cl_2C_6H_3S$–	0.12	1.2
10	NH_2	OH	2-$C_{10}H_7SO$–	0.042	5.3
11	NH_2	OH	2-$C_{10}H_7SO_2$–	0.011	0.50
12	NH_2	OH	3,4-$Cl_2C_6H_3SO_2$–	0.076	5.0
13	NH_2	SH	2-$C_{10}H_7S$–	0.23	0.80
14	NH_2	SH	2-$C_{10}H_7SO_2$–	0.025	6.8
15	OH	NH_2	2-$C_{10}H_7S$–	1.7	46.0
16	SH	NH_2	2-$C_{10}H_7S$–	3.0	20.6
17	H	NH_2	2-$C_{10}H_7S$–	21.0	85.3
18	H	NH_2	2-$C_{10}H_7SO$–	15.0	26.1
19	H	NH_2	2-$C_{10}H_7SO_2$–	3.4	312[e]
20	OH	OH	2-$C_{10}H_7S$–	8.6	48.4
21	SH	OH	2-$C_{10}H_7S$–	10.0	141
22	OH	SH	2-$C_{10}H_7S$–	5.8	57.0
23	SH	SH	2-$C_{10}H_7S$–	8.9	222[e]
24	CH_3CONH	OH	2-$C_{10}H_7SO_2$–	0.78	555[e]
Pyrimethamine[d]				0.070[f]	3.0[g]
Trimethoprim[d]				24[f]	0.022[g]

[a] Assayed spectrophotometrically at 340 mμ. [b] Conditions: dihydrofolate, 9 μM; NADPH, 30 μM; KCl, 0.15 M; in 0.05 M Tris buffer (pH 7.4). [c] Conditions: dihydrofolate, 30 μM; NADPH, 50 μM; KPO_4, 0.05 M (pH 5.6).[12] [d] Sample kindly provided by Division of Medicinal Chemistry, Walter Reed Army Institute of Research. [e] By extrapolation due to limited solubility. [f] R. Ferone, J. J. Burchall, and G. H. Hitchings [*Mol. Pharmacol.*, **5**, 49(1969)] reported 0.7 μM for pyrimethamine and 250 μM for trimethoprim using 50 μM dihydrofolate. [g] J. H. Freisheim, *et al.*[12]

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.

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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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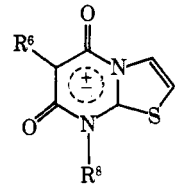
The discovery of *in vitro* antibacterial activity of mesoionic thiazolo[3,2-*a*]pyrimidine-5,7-diones (**1**) and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones (**2**) has been recently reported.[1,‡] These compounds are members of a large, virtually unknown, class of mesoionic structures, 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.



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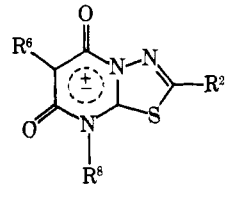

2

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.