of **17** and 250 mg (2 mmoles) of DBN in 2 ml of DMF cooled in an ice bath at −10° was added 220 mg (1 mmole) of *m*-fluorosulfonylbenzoyl chloride over about 5 min with stirring. After 15 min the solution was poured into a stirred mixture of 30 ml of 1 *N* H_2SO_4 and 10 ml of $CHCl_3$. The collected product was washed with hot $CHCl_3$, then recrystallized from glacial HOAc containing a few drops of 6 *N* H_2SO_4; yield 215 mg (64%), gradually decomposed over 150° and moved as one spot on tlc in 1:4 EtOH–$CHCl_3$. *Anal.* ($C_{21}H_{22}FN_5O_3S \cdot H_2SO_4 \cdot 0.5H_2O$) C, H, F.

2,4-Diamino-5-[*p*-(*m*-fluorosulfonylbenzamido)phenylbutyl]-6-methylpyrimidine (3) Hemisulfate.—Reaction of 420 mg (1 mmole) of crude **11**·1.5H_2SO_4 with 220 mg (1 mmole) of acid chloride, as described for **4**, gave a crude product that was recrystallized from EtOH–H_2O; yield 90 mg (17%), mp >161° with gradual decomposition. *Anal.* ($C_{22}H_{24}FN_5O_3S \cdot 0.5H_2SO_4 \cdot H_2O$) C, H, F.

The *p*-benzamide (**18**) was prepared as described for **4**; yield 130 mg (38%), mp 211–218° dec. *Anal.* ($C_{21}H_{22}FN_5O_3S \cdot H_2SO_4 \cdot H_2O$) C, H, F.

By reaction of **17** with O-(*p*-nitrophenyl) N-(*p*-fluorosulfonylphenyl)carbamate,[14] as described for the preparation of **4**, was obtained **19** in 46% yield, mp >140° with gradual decomposition. *Anal.* ($C_{21}H_{23}FN_6O_3S \cdot H_2SO_4 \cdot 0.5H_2O$) C, H, F.

Enzyme Results and Discussion

Replacement of the ether linkage in the bridge of **1** by methylene (**3**) gave little change in the ability of the compound to inactivate mouse L1210 dihydrofolic reductase, nor was specificity changed since **3** showed no significant inactivation of the mouse liver enzyme (Table I). Unfortunately, penetration through the L1210 cell wall was still poor with **3** since there was little change in ED_{50}[16] or the normalized ED_{50}/I_{50} compared to **1**. The ED_{50}/I_{50} = 68 for **3** should be compared with the ED_{50}/I_{50} = 0.003 for **2**.[3,5]

When the 6-methyl group of **3** was replaced by H, the resultant **4** was still an excellent irreversible inhibitor of L1210 dihydrofolic reductase, but showed perceptible inactivation of the mouse liver enzyme; however, **4** was even less effective than **3** against intact L1210 cells in culture. Even though **18** and **19** were less effective on the L1210 enzyme and less specific than **4**, these two compounds were assayed against L1210 cell culture; again, penetration was poor.

Since such high specificity against L1210 dihydrofolic reductase is obtained with **1** and **3**, further studies would be warranted to see if cell penetration can be improved. Variants at the 6 position of the pyrimidine, the oxypropyl bridge between the pyrimidine and inside phenyl, as well as the bridge between the two benzene rings are under continued investigation.

(16) We wish to thank Dr. Florence White of the CCNSC for the L1210 cell culture data.

2,4-Diaminopyrimidines. The Cyclization of 6-Phenacylthio and Related Derivatives to Thieno[2,3-*d*]pyrimidines and Thiazolo[3,2-*c*]pyrimidines[1]

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2,4-Diamino-5- and -6-substituted thieno[2,3-*d*]pyrimidines have been prepared from 2,4-diamino-6-mercaptopyrimidine plus α-halo ketones. The ease of cyclization of the intermediate pyrimidyl sulfides (Pyr-SCHR'COR) varies dramatically with the R and R' substituents. When R = *p*-bromophenyl and R' = H, cyclization can be effected in low yield at 200° in inert medium. On the other hand, with R = methyl and R' = benzyl, cyclization proceeds spontaneously at room temperature in slightly acidic medium. In concentrated sulfuric acid, where R = *p*-bromophenyl and R' = H, the isomeric thiazolo[3,2-*c*]pyrimidinium sulfate is readily produced. This compound is stable only as the cation. In alkali, the pyrimidine ring opens with loss of its 2-carbon atom. The 2,4-diaminothieno[2,3-*d*]pyrimidines are weak bases, with pK_a values below 5. A bulky R' group and small R substituent favors activity as a dihydrofolate reductase inhibitor, but slightly acidic solutions are required for maximum activity. The low pK_a values of these compounds militate against wide utility, since the protonated species is required for enzyme binding.

Our laboratories have been engaged for many years in chemotherapy studies based on the inhibition of folate biosynthesis and function.[2] Many derivatives of 2,4-diaminopyrimidine have been found to be potent inhibitors of the enzyme dihydrofolate reductase, which plays a major role in folic metabolism by catalyzing the reduction of dihydrofolate to its active cofactor form, tetrahydrofolate. This cofactor is involved in at least 15 biosynthetic transfer reactions of one-carbon fragments involved in amino acid and nucleic acid synthesis.[3]

Impetus to the search for new compounds which block the action of this enzyme has been given by the finding that dihydroreductases from microbial *vs.* mammalian sources differ greatly in their binding capacity for different diaminopyrimidines and related compounds.[4] For example, the antibacterial agent trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine][5] is bound 50,000 times more strongly to bacterial than to mammalian enzymes; this provides a sound explanation for its therapeutic effectiveness.[6]

(1) This paper was presented in part at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966.

(2) See, for example, G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 416 (1965), and references cited therein.

(3) (a) M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963); (b) T. H. Jukes and H. P. Broquist in "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963 pp 481–534.

(4) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965).

(5) B. Roth, E. A. Falco, G. H. Hitchings, and S. R. M. Bushby *J. Med. Pharm. Chem.*, **5**, 1103 (1962).

(6) (a) J. H. Darrell, L. P. Garrod, and P. M. Waterworth, *J. Clin. Pathol.*, **21**, 202 (1968); (b) A. S. E. Fowle, C. D. M. Drew, D. T. D. Hughes and M. A. Cassell, *Proc. Intern. Congr. Chemotherapy, 5th, 1967*, **1**, 293 (1967); (c) B. W. Csonka and G. J. Knight, *Brit. J. Venereal Diseases*, **43**, 161 (1967); (d) M. Schneider, L. Schwarzenberg, A. Cattan, J. R. Schlumberger, J. L. Amiel, and G. Mathe, *Presse Méd.*, **73**, 893 (1965); (e) R. G. Cooper and M. Wald, *Med. J. Aust.*, **2**, 93 (1964); (f) E. W. P. Noall, H. F. G. Sewards, and P. M. Waterworth, *Brit. Med. J.*, **2**, 1101 (1962); (g) *ibid.*, **2**, 380 (1968); (h) S. R. M. Bushby and G. H. Hitchings, *Brit. J. Pharmacol.*, **33**, 72 (1968)

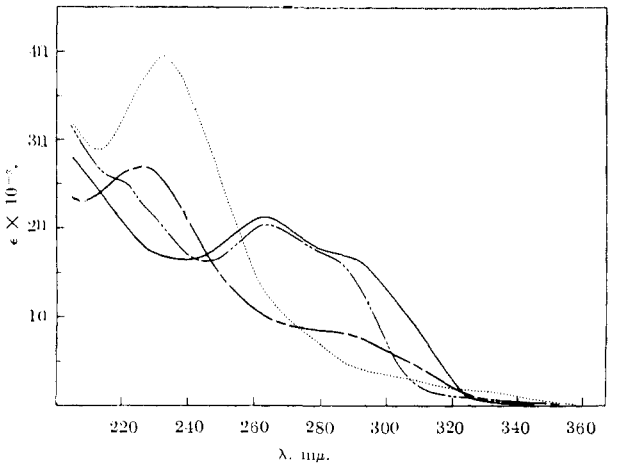
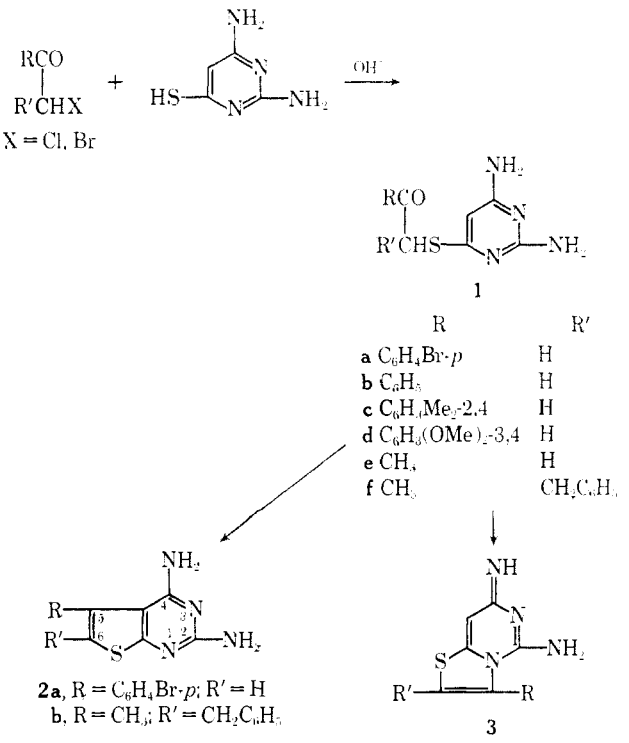


Figure 1.—Uv spectra of **1a** and **2a**: neutral species **1a**, ——; cation **1a**, ——; neutral species **2a**, — — —; cation **2a**, · · · · · ·.

SCHEME I



The thieno[2,3-*d*]pyrimidine ring system, which is isosteric with that of the known active diaminoquinazolines,[7] has not previously been examined with regard to dihydrofolate reductase activity. The first synthesis of a thieno[2,3-*d*]pyrimidine was carried out by Baker and coworkers,[8] in studies of analogs of the hydrangea alkaloids. Thiophene intermediates were used in their synthesis. Taylor and Berger[9] prepared several 4-amino-5,6-substituted thieno[2,3-*d*]pyrimidines from 2-amino-3-cyanothiophenes. A recent dissertation by Chacko[10] reports similar syntheses. Shvedov and coworkers[11] have also prepared related compounds from 2-amino-3-carbethoxythiophenes. In no case has a pyrimidine been used as the intermediate.

It appeared that the desired 2,4-diaminothieno[2,3-*d*]pyrimidine system **2** might be obtained from the readily available 2,4-diamino-6-mercaptopyrimidine[12] by reaction with α-halo ketones, *via* an intermediate pyrimidyl sulfide derivative **1** (Scheme I). Phenacyl bromides were found to react readily with the mercaptopyrimidine (see Experimental Section) to yield the corresponding 6-phenacylthiopyrimidines in high yield. Chloroacetone reacted similarly with the pyrimidine. No cyclization occurred under the conditions used, nor with an excess of base.

Various conditions were tried for the cyclization, and it was found that the *p*-bromophenacylthio derivative **1a** could be cyclized to the desired thieno-[2,3-*d*]pyrimidine **2a** in low yield by heating the intermediate in an indifferent solvent (see Experimental Section). The temperature required for the cyclization was critical, and unfortunately also resulted in decomposition of most of the material to low molecular weight by-products. Under corresponding conditions, the *o,p*-dimethylphenacylthio analog **1c** was recovered unchanged, and at 240° the portion which reacted consisted of black tarry material. The other phenacylthio analogs produced tars and low molecular weight decomposition products in the 200–210° region, and no thienopyrimidine was isolated.

It was possible that the cyclization of type **1** compounds occurred on the 1-nitrogen of the pyrimidine ring instead of the 5-carbon, to produce thiazolo[3,2-*c*]pyrimidinimines (**3**). Indication that the cyclization had proceeded in the desired sense, and not on the 1-nitrogen, was provided by the pmr spectrum of **2a**, which showed one aromatic singlet (1 H) in addition to the benzene signals. Cyclization to produce **3a** would have produced a product with two aromatic singlets in addition to the benzene doublets.

The uv spectra of **1a** and **2a** are shown in Figure 1 as the neutral and cationic species. The maximum at 263 mμ for **1a** is due to the aromatic ketone absorption; this peak is lost on cyclization. The large hyperchromic shift of the cationic species upon formation of the bicyclic system is also to be noted. The thermodynamic pK_a for **1a** was found to be 4.98 at 20°.[13] The dissociation constant for the cyclized product **2a** is 4.47.[13] This decrease in pK_a is to be expected with the introduction of the vinyl group in the pyrimidine 5 position. A compound of type **3**, however, would be expected to be a strong base.

The intermediate **1a** was found to be insoluble in dilute aqueous acid; no reaction occurred on refluxing for several hours in dilute ethanolic HCl. It dissolved readily in concentrated H_2SO_4 without undergoing change at room temperature. However, at 100° a new compound (**4**) was formed, with an elementary analysis which corresponded to the sulfate salt of a cyclized derivative. The uv spectrum of this sub-

(7) G. H. Hitchings, E. A. Falco, and K. W. Ledig, U. S. Patent 2,945,859 (1960).

(8) B. R. Baker, J. P. Joseph, R. E. Schaub, F. J. McEvoy, and J. H. Williams, *J. Org. Chem.*, **18**, 138 (1953).

(9) E. C. Taylor and J. G. Berger, *Angew. Chem. Intern. Ed. Engl.*, **5**, 131 (1966).

(10) A. M. Chacko, *Dissertation Abstr.*, **26**, 3627 (1966).

(11) V. I. Shvedov, V. K. Ryzhkova, and A. N. Grinev, *Khim. Geterotsikl. Soedin.*, 359 (1967).

(12) G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 2858 (1956).

(13) B. Roth and J. Z. Strelitz, *J. Org. Chem.*, in press.

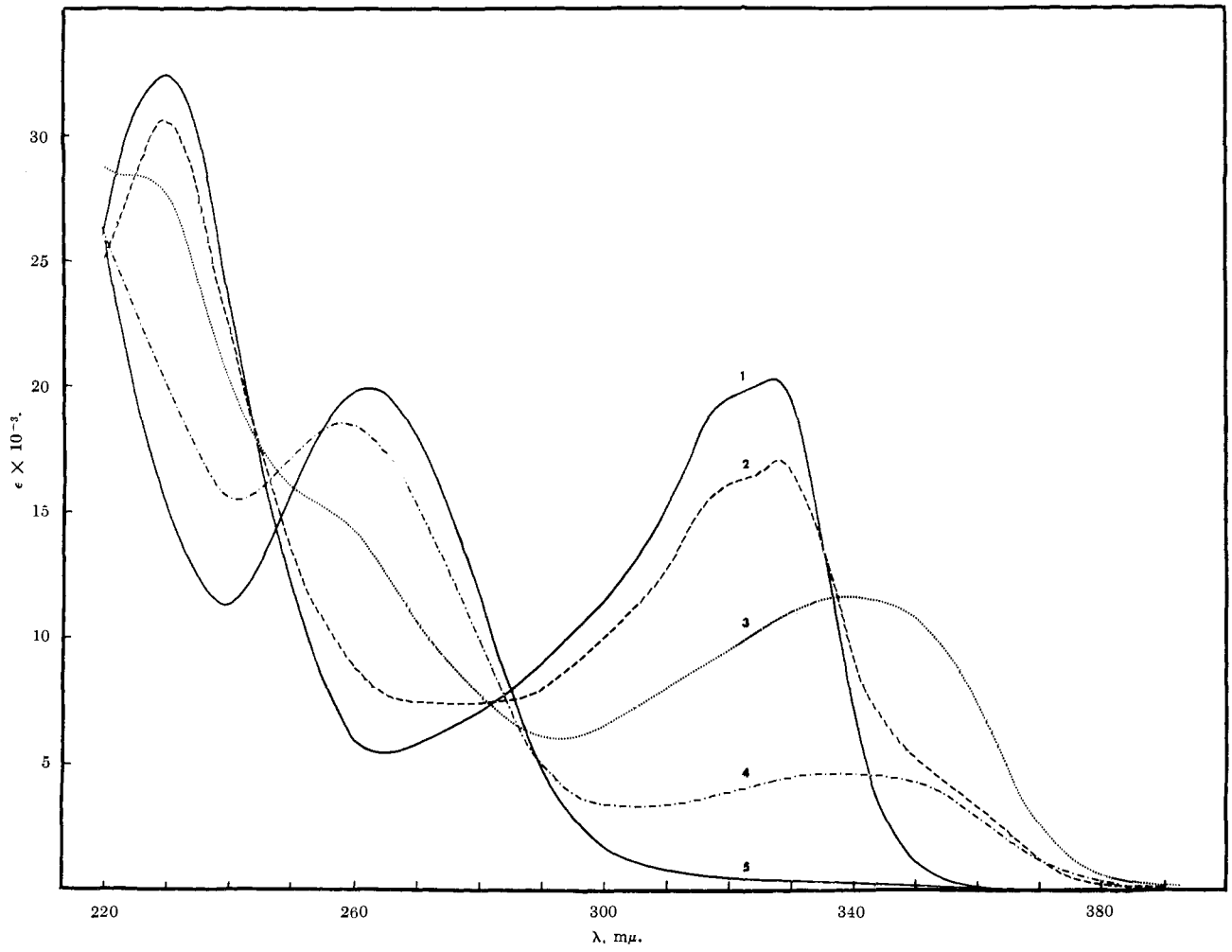
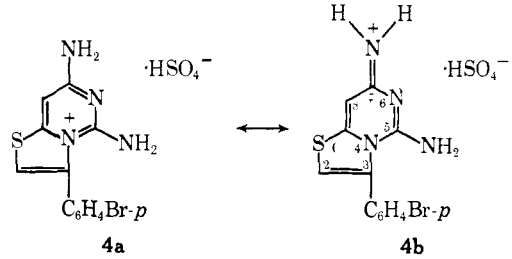


Figure 2.—Uv spectra of **4** and alkali degradation products: 1, in 0.1 *N* HCl; 2, at pH 11.6 (0.1 *N* glycine–NaOH); 3, in 0.1 *N* NaOH initial spectrum, 20°; 4, in 0.1 *N* NaOH, 32 min, 20°; 5, in 0.1 *N* NaOH, 19 hr, 20°.

stance, shown in Figure 2 (curves 1 and 3), is entirely different from that of **2a**. This spectrum did not change between pH 0 and 10, but a pK_a was found at approximately pH 11.5. However, the molecule was very unstable at pH values above 11 at room temperature, as evidenced by rapid changes in the uv spectrum, also shown in Figure 2. The half-time for the decomposition at 22°, measured at 340 mμ, was found to be *ca.* 1700 sec.

The pmr spectra of the sulfate in DMSO-d_6 and in DMSO plus D_2O are shown in Figure 3. These spectra indicate that cyclization occurred on the pyrimidine N^1 to produce a thiazolo[3,2-*c*]pyrimidinium sulfate, which is probably protonated as shown in structures **4a** and **4b**.



4a **4b**

In addition to the benzene doublets, there are two sharp singlets for the 2 and 8 protons. The upfield amino signal appears as a broad singlet (2 H), but the other is split into two NH peaks, indicating that it exists in a partially doubly bonded coplanar configuration (**4b**), with nonequivalent nitrogens. The fact that these signals are not at extremely high field would indicate that a fairly large portion of the positive charge resides on the 4-nitrogen atom at the ring juncture. Such a structure would be expected to be a strong base, as indeed it is.

Andrew and Bradsher[14] have recently reported a similar type of cyclization with 2-phenacylthio-4-pyrimidones in concentrated H_2SO_4. No mention is made of instability in alkali in their paper.

Ring N-alkylated 2- and 4-iminopyrimidines are very much stronger bases than their nonalkylated counterparts (*cf.* 1-methyl-2-pyrimidoninine, pK_a = 10.7, *vs.* 2-aminopyrimidine, pK_a = 3.54).[15] Furthermore, the N-alkylated derivatives are unstable in alkali. In cases where the imino group is adjacent to the N-alkyl group, rearrangement to alkylamino derivatives occurs by way of ring opening.[16] Such facts are also consistent with structure **4**.

Treatment of **4** with 0.1 *N* NaOH produced a white

(14) H. F. Andrew and C. K. Bradsher, *J. Heterocycl. Chem.*, **4**, 577 (1967).
(15) See D. J. Brown, "The Pyrimidines," John Wiley and Sons, Inc., New York, N. Y., 1962, pp 472–473.
(16) D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1276 (1963).

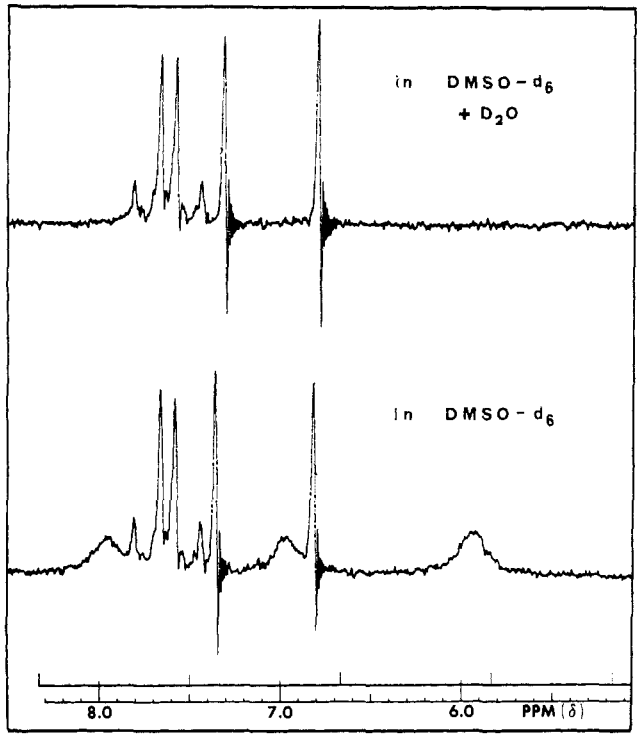
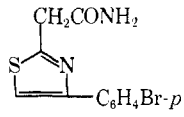


Figure 3.—Pmr spectrum of **4**.

crystalline substance, which was a mixture of two closely related products. The pmr spectrum (see Experimental Section) showed one vinyl proton in addition to the benzene peaks, and a CH_2 group. The additional absorption appeared to be a mixture of amine-type peaks. The uv spectrum showed a single maximum at 262 mμ as the neutral species, with a pK_a in the vicinity of 2. These facts, coupled with the analysis, indicated degradation to the thiazoloacetamide **5**, accompanied by approximately 25% of the corresponding amidine. The known 2-methyl-4-(*p*-



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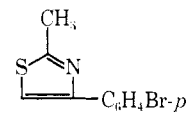
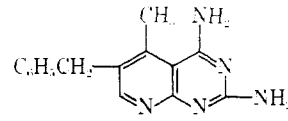


6

bromophenyl)thiazole (**6**)[17] was prepared. The pmr spectrum (DMSO-d_6) showed a CH_3 singlet at 2.71 (δ), an AB pattern of benzene doublets centered at 7.57 and 7.86 (J = 8.5 Hz), and a singlet at 7.91 ppm for the 5-thiazole proton. The uv spectrum was almost identical with that of **5**, and the pK_a was 2.2, all of which is confirmatory for structure **5**. This reaction appears to take place by attack of OH^- at the 5 position of the thiazolopyrimidine, causing ring opening between the 4 and 5 positions of **4** base, and donation of the electrons to the positive N^+ at the ring juncture. Further degradation of the open-chain fragment in alkali would lead to the amidine, amide, and probably other products which were not isolated.

The thienopyrimidine **2a** was found to have quite low activity in an *in vitro* antibacterial screen. This was not considered surprising, since in most related bicyclic systems which have been prepared, maximum activity has been obtained with a small substituent in the position corresponding to C-5 of **2** and a heavy substituent in the related 6 position.[18] It appeared that biological activity, as well as the synthesis, might be improved by using a ketone with a small R and large R′ group. Such substitution would be expected to favor ring closure, rather than offering steric resistance. To test this hypothesis, benzylacetone was brominated to yield 3-bromo-4-phenyl-2-butanone (**7**). The thermodynamic prediction that the reaction product of **7** with 2,4-diamino-6-mercaptopyrimidine should cyclize with greater ease proved to be astonishingly accurate, in that the cyclized product **2b** was obtained directly in high yield on warming the two ingredients together with 1 mole of alkali, with the provision that **7** was pure. When this was not the case, and the final reaction mixture was basic, an intermediate was isolated which appeared to be a hydrated form of the intermediate pyrimidyl sulfide, but, upon acidification, this intermediate was spontaneously converted to the cyclic derivative **2b**. We found no evidence of partial bromination of benzylacetone on the methyl group, from pmr investigation of the intermediate and final products. Impurities were caused by loss of HBr from the rather unstable **7**. That the cyclized product was indeed **2b** as shown, was indicated by the pmr spectrum, which showed only methyl, methylene, phenyl, and amino signals. The thermodynamic pK_a of the product (20°) was 4.90,[18] which gave further indication that the compound had cyclized in the desired sense, and not on N′.

Thienopyrimidine (**2b**) is isosteric with 2,4-diamino-5-methyl-6-benzylpyrido[2,3-*d*]pyrimidine (**8**),[19] which has been found to be a very potent antibacterial agent.[1]



8

The chief difference between the two compounds lies in their pK_a values, which are about 2 units apart (6.98 for **8**).[18] The thienopyrimidine was found to have high inhibitory activity against *Lactobacillus casei*, an acid-forming bacterium. In OFA medium, the compound showed 89% inhibition at 0.05 μg/ml, and 41% inhibition at the same level in FA+ medium, whereas **2a** showed only 26% inhibition in OFA medium at 100 μg/ml.[20] However, in plating tests against a variety of other bacteria in neutral agar, no inhibitory zones were seen with **2a** or **2b**, except for slight activity against *Streptococcus faecalis* with **2b**. Compounds **2b** and **8** were then compared against isolated enzyme systems from two sources, at two pH values. The data are shown in Table I.

The thienopyrimidine is more active against both bacterial and mammalian enzymes at the lower pH

(17) J. P. Wetherill and R. M. Hann, *J. Am. Chem. Soc.*, **56**, 970 (1934).

(18) G. H. Hitchings, T. A. Herrmann, B. S. Hurlbert, and S. R. M. Bushby, *Proc. Intern. Congr. Chemotherapy, 3rd, Stuttgart*, 1963, 1363 (1965).

(19) B. S. Hurlbert, K. Lelig, P. Steinbeck, B. Valeno, and G. H. Hitchings, *J. Med. Chem.*, **11**, 703 (1968).

(20) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Shoowood, and H. VanderWerff, *J. Biol. Chem.* **183**, 1 (1950).

TABLE I

COMPARATIVE BINDING OF ISOSTERIC THIENO- AND PYRIDOPYRIMIDINES BY BACTERIAL AND MAMMALIAN DIHYDROFOLATE REDUCTASES AT TWO pH VALUES[a]

Enzyme source	pH	Concn for 50% inhib. $M \times 10^8$ 2b	8
E. coli	7	18	1.0
E. coli	5.5	2.3	2.5
Rat liver	7	1000	4.0
Rat liver	5.5	23	25

[a] The protocol was similar to that of ref 4.

value, by factors of about 8 and 50, respectively. The reverse is seen to be true with **8**, which is more active at neutral pH. It is interesting that at pH 5.5 both compounds have virtually identical activities against both enzymes, and at a level which is therapeutically useful. At pH 7, the pyridopyrimidine is considerably more active in both cases; however, the thienopyrimidine has a much better therapeutic index against the bacterial enzyme than does the pyridopyrimidine. Of course we do not have complete data as to pH optima here. However, the data give evidence not only that the protonated forms of the pyrimidines are involved in binding to the enzymes, but that there are other subtle differences between these two compounds which lead to differential binding between enzymes from different sources; these presumably have slightly different amino acid sequences.

Experimental Section[21]

2,4-Diamino-6-(*p*-bromophenacylthio)pyrimidine (1a).—A mixture of 14.2 g (0.1 mole) of 2,4-diamino-6-mercaptopyrimidine, 6.0 g (0.11 mole) of NaOMe, and 180 ml of $(CH_2OH)_2$ was heated with stirring until a clear solution was obtained (80°). Then 27.8 g (0.1 mole) of *p*-bromophenacyl bromide was added. An immediate red color developed, followed by the formation of a white precipitate. The solution was heated at 120° for 30 min. After cooling, the precipitate was isolated and washed (EtOH, H_2O): 15.4 g (dry). Dilution of the glycol filtrate with H_2O precipitated an additional 15.0 g of crude product (90% total). The fractions were each recrystallized from Me_2CO–H_2O (320:50 ml) with Darco G-60. Recovery was 12.5 and 8.4 g, respectively, of identical large off-white plates. Further recrystallization yielded a white product: mp 199–200°; pmr spectrum (DMSO-d_6), δ 4.66 (CH_2), 5.73 (H_5), 5.90 (NH_2), 6.28 (NH_2), 7.76 and 8.00 ppm (doublets, J = 8.5 Hz) (*p*-C_6H_4); uv spectrum, see Figure 1 and ref 13. Characteristic ir bands (KBr) were present at 3425 (s), 3215 (m), 1686 (m), 1631 (s), 1572 (s), 1433 (m), 1381 (m), and 1282 (m) cm^{-1}. *Anal.* ($C_{12}H_{11}BrN_4OS$) C, H, N.

2,4-Diamino-6-phenacylthiopyrimidine (1b) was prepared in the same manner as **1a**; mp 138–139° (95% EtOH). *Anal.* ($C_{12}H_{12}N_4OS$) C, H, N.

2,4-Diamino-6-(2,4-dimethylphenacylthio)pyrimidine (1c).—A similar preparation starting from 2',4'-dimethylphenacyl chloride, followed by recrystallization from 45% EtOH, produced white crystals, mp 173–175°. *Anal.* ($C_{14}H_{16}N_4OS$) C, H, N.

2,4-Diamino-6-(3,4-dimethoxyphenacylthio)pyrimidine (1d) was prepared similarly, from 3',4'-dimethoxyphenacyl bromide. The substance was very insoluble in alcohols and acetone. Recrystallization from DMF–H_2O (90:10) produced white crystals: mp 248–249° dec; pmr spectrum (DMSO-d_6), δ 3.82 (OCH_3), 3.87 (OCH_3), 4.64 (CH_2), 5.70 (H_5), 5.96 (NH_2), 6.23 (NH_2), 7.08 (doublet, J = 8.5 Hz) (Bz H_5), 7.53 (doublet, J = 2 Hz) (Bz H_2), 7.74 (quadruplet, J = 8.5 and 2 Hz) (Bz H_6) ppm. *Anal.* ($C_{14}H_{16}N_4O_3S$) C, H; N: calcd, 17.54; found, 17.07.

2,4-Diamino-6-acetonylthiopyrimidine (1e).—To 7.1 g (0.05 mole) of 2,4-diamino-6-mercaptopyrimidine in 50 ml of 1 *N* NaOH was added 4.62 g (0.05 mole) of chloroacetone. The resulting orange solution was allowed to stand for 15 min at 40° and then heated on the steam bath for 3 min, which caused considerable darkening of the solution. Slow chilling produced a black oil that eventually crystallized, 6.23 g (63%). Repeated recrystallization from EtOH (with Darco G-60) produced light crystals, mp 146–147°. *Anal.* ($C_7H_{10}N_4OS$) C, H, N.

2,4-Diamino-5-(*p*-bromophenyl)thieno[2,3-*d*]pyrimidine (2a). A mixture of 10 g of 2,4-diamino-6-(*p*-bromophenacylthio)-pyrimidine and 60 ml of $(C_6H_5)_2O$ was heated in an oil bath to 210°. A clear yellow solution formed at 160°, which darkened and formed a slight precipitate at 200°. After 5 min at 205–210°, the mixture was allowed to stand at room temperature overnight. The brown precipitate was isolated and washed with Et_2O; 5.62 g (dry). This material consisted mainly of degradation products which were easily soluble in hot EtOH.

Hexane was added to the $(C_6H_5)_2O$ filtrate and a tan precipitate separated, 2.77 g (dry). This was extracted with 20 ml of 95% EtOH at room temperature. The insoluble portion (1.35 g) was recrystallized three times from 95% EtOH (1 g/150 ml), which yielded 352 mg of off-white crystals, mp 224–225°. The estimated total yield of the product, from spectral determinations of various fractions, was about 10%. *Anal.* ($C_{12}H_9BrN_4S$) C, H, N, S; Br: calcd, 24.88; found, 25.44.

The pmr spectrum (TFA) showed an AB pattern of doublets at δ 7.45 and 7.83 ppm (J = 8.5 Hz) (*p*-C_6H_4) with a singlet (H_6) superimposed on the upfield doublet at 7.35. The amine signals were not visible. In DMSO-d_6, amine signals were present at δ 5.78 and 6.15, along with a singlet at 6.84 (H_6) and benzene doublets at 7.36 and 7.64 ppm (J = 8.5 Hz). The uv spectrum[13] is pictured in Figure 1.

The temperature and time for this thermal cyclization were very critical. At 190–195°, starting material was recovered for the most part. At 220° and above, only decomposition products were isolated.

3-(*p*-Bromophenyl)-5,7-diaminothiazolo[3,2-*c*]pyrimidinium Sulfate. (4).—2,4-Diamino-6-(*p*-bromophenacylthio)pyrimidine (4 g) was mixed with 24 ml of concentrated H_2SO_4. The clear colorless solution was heated at 100° for 2 hr in a flask protected from moisture. The solution was chilled and poured on ice. The resultant gum soon crystallized and was isolated, washed (ice–water, Et_2O), and vacuum dried: 3.3 g. Recrystallization from 60 ml of 0.55 *M* H_2SO_4 (with Darco G-60), followed by chilling overnight, produced a white crystalline sulfate salt (2.1 g): pmr spectrum (DMSO-d_6), δ 7.74 and 7.59 (d, J = 8.5 Hz) (*p*-C_6H_4), 7.42 (s), 6.87 (s), 8.52 (NH), 8.00 (NH), and 7.02 (NH_2) (see Figure 3); uv spectrum, $\lambda_{max}^{0.1\ N\ HCl}$ 230 mμ (ε 30,800), 328 (17,600) (see also Figure 2). *Anal.* ($C_{12}H_9BrN_4S \cdot H_2SO_4$) C, H, N, S.

The filtrate from the crystallized product was adjusted to pH 13 with NaOH. An incipient white precipitate soon redissolved for the most part and the solution turned yellow. Heating on the steam bath for 10 min resulted in separation of a greenish yellow precipitate. This was isolated, washed (H_2O), and dried: 0.37 g. The substance was recrystallized from 7 ml of EtOH. A small amount of greenish insoluble material was separated, and after Darco treatment, 265 mg of shiny creamy crystals were isolated. Two more recrystallizations produced shiny white plates, mp 164–165°. The analytical, spectral, and chromatographic data indicated this to be a mixture consisting of 75% of 2-carboxamidomethyl-4-(*p*-bromophenyl)thiazole (**5**) and 25% of the corresponding amidine; pmr spectrum (DMSO-d_6), δ 3.97 (s) (CH_2), 7.63 and 7.91 (d, J = 8.5 Hz) (*p*-C_6H_4), 8.04 (s) (C_5), plus small broad NH signals centered at 5.73 and 6.15 ppm, and an additional very broad NH signal in the benzene region (total NH integration slightly greater than 2 H); uv spectrum (neutral species), $\lambda_{max}^{pH\ 6}$ 262 mμ (ε 19,200), $\lambda_{max}^{pH\ 1}$ 257 (20,200), λ_{max} (H_0 = −0.2) 255 (21,000). The changes in spectrum between pH 4 and 0 indicated that more than two species were present, since the curves deviated from an isobestic point. The major component was estimated to have a pK_a value of approximately 2. The ir spectrum (KBr) showed characteristic bands at 3378 (s), 3185 (m), 1669 (s), 1634 (m), 1492 (m), 1404 (m), 1193 (w), 1011 (m), 830.6 (m), and 750.8 (m) cm^{-1}. *Anal.* Calcd for ($C_{6}H_9BrN_2OS$ (amide **5**): C, 44.46; H, 3.05; N, 9.43; S, 10.79. Found: C, 44.42; H, 3.15; N, 10.60; S, 10.92 (calcd for amidine: N, 14.19).

(21) Melting points are corrected and were determined with a hot stage microscope. Where analyses are indicated only by symbols of the elements or functions, analytical results for those elements or functions were within ±0.4% of the theoretical values.

2,4-Diamino-5-methyl-6-benzylthieno[2,3-*d*]pyrimidine (2b).—Benzylacetone was brominated in cold $CHCl_3$ by the method of Janetzky and Verkade,[22] and vacuum distilled twice using a 20-cm vacuum-sealed Vigreux column. The fraction boiling at 88–92° (0.6–0.8 mm) was used for reaction with the pyrimidine. A mixture of 32.6 g (0.23 mole) of 2,4-diamino-6-mercaptopyrimidine, 12 g (0.23 mole) of NaOMe, and 300 ml of $(CH_2OH)_2$ was heated to 80° with stirring, at which point a clear solution was present. The above bromo ketone (52 g) was then added, and the mixture was heated at 110° for 30 min. A clear dark red solution formed. After cooling, this was poured into H_2O; the resultant pH was about 6. The solid which precipitated was collected and recrystallized directly from 95% EtOH; 39 g (63% calculated as **2b**). Further recrystallization from EtOH (*ca.* 75 ml/g) produced white needles: mp 231–232°; pmr spectrum (DMSO-d_6), δ 2.43 (CH_3), 4.04 (CH_2), 5.95 (NH_2), 6.42 (NH_2), 7.27 (C_6H_5); uv spectrum, see ref 13. *Anal.* ($C_{14}H_{14}N_4S$) C, H, N, S.

The bromination product of benzylacetone, 3-bromo-4-phenyl-2-butanone (**7**), was found to be quite unstable. In some preparations, the crude bromo ketone was used directly in the next reaction, but in all cases this led to mixtures. In many cases, the distilled fractions were found to consist of mixtures of two and three products, as determined by their pmr spectra and by tlc. In a typical case which showed two other components to be present to the extent of 25–30%, the bromo ketone was treated directly with the mercaptopyrimidine as above, calculated on the basis that the bromo ketone was pure. At the end of the reaction, after pouring the mixture into H_2O, the solution was quite alkaline, and the product separated as a gum. This was extracted (warm H_2O, Et_2O–EtOAc). A white solid resulted which had a pmr spectrum with two sharp benzene singlets at 7.27 and 7.30 ppm (δ) in a ratio of 7:1, which appeared to correspond to a 7:1 ratio of uncyclized to cyclized product. The uncyclized portion was evidenced by a sharp singlet at 5.75 ppm which integrated for one-fifth of the large benzene singlet (pyr 5-H), broad singlets at 6.08 and 6.01 (2 NH_2), a doublet and triplet at 3.97 and 3.35, respectively (J = 9 Hz) (–$CHCH_2$–), a broad singlet at 1.35 (CH_3), and broad singlets at 2.98, 2.78, and 1.57 ppm, each integrating for about one-half a proton, plus small additional absorption in the 2.5–4 region. The location of the methyl peak indicated that it was not next to a keto function; possibly the ketone was hydrated or partially converted to an acetal.

The uv spectra of this substance in neutral and alkaline solutions were quite different from that of the cyclized product, and the lack of isosbestic points indicated that a mixture was present. When the solution was acidified, the spectrum immediately became the same as that of the cyclized product. A 0.5-g sample of the substance was slurried in 20 ml of 0.1 *N* HCl. Most of it dissolved, and then a heavy precipitate formed, which remained insoluble upon heating the mixture to the boiling point. The precipitate was isolated, treated with alkali to reconvert it to the free base, and recrystallized from ethanol. The properties were now identical with those of the first-described cyclized product (**2b**).

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Tumor Localizing Agents. VII.[1] Radioiodinated Quinoline Derivatives

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Several radioiodinated analogs of chloroquine were synthesized in an effort to find an agent which would selectively localize in melanomas. One compound, 4-(3-dimethylaminopropylamino)-7-iodoquinoline-^{125}I was found to have a marked affinity for melanin-containing tissues and for melanotic tumors in animals.

While malignant melanoma is only one of the many varieties of malignant tumors, its appearance and manifest virulence has stimulated study over the ages. It comprises about 2% of all cancers and about 80–90% of them arise in the skin.[2] Although no age group is immune, the majority of melanomas arise in persons 31–60 years of age and there appears to be no sex predilection.[2] Despite its low incidence, Raven[3] has emphasized the urgent need for new agents which will assist the physician in diagnosing the metastic nature of the tumor. The early recognition of the tumor before dissemination occurs, as well as determining the spread of the tumor once metastases have appeared, is important for determining the course of treatment and survival of the patient.

As part of a broad program aimed at the development of an agent which would be useful for the diagnostic localization and treatment of melanotic tumors, studies in our laboratory have concentrated on finding radiolabeled compounds that would selectively concentrate in these tumors. Such compounds labeled with γ-emitting radionuclides could serve as diagnostic agents when used in conjunction with external scintillation scanners and cameras. Moreover, it is possible that the same compounds could be employed therapeutically when the radionuclide decays with emission of β rays.

In order to achieve the essential selective localization in melanomas, our studies have fallen into two categories, namely, (a) precursors of melanin[4] and (b) compounds which are known to interact with melanin.[5] Although our results to date with radiolabeled melanin precursors have been discouraging, preliminary studies

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