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2,4-Diaminothieno[2,3-*d*]pyrimidines as Antifolates and Antimalarials. 2. Synthesis of 2,4-Diaminopyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidines and 2,4-Diamino-8*H*-thiopyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidines†

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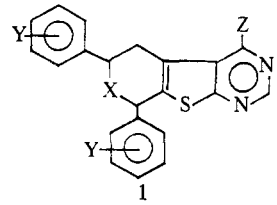
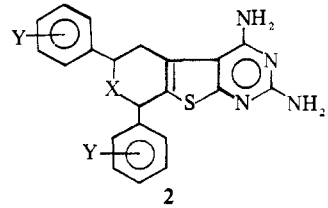
Several new 2,4-diamino-6,8-diaryl-5,6-dihydro-8*H*-thiopyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidines, including some sulfoxide and sulfone derivatives, were synthesized as candidate antifolate antimalarials. One example of the 2,4-diamino-6,8-diaryl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidine type was likewise prepared. The key synthetic step involved condensation of the appropriate 2-amino-3-cyanothiophene intermediate with chloroformamidine hydrochloride, a useful reagent for the formation of ring-fused 2,4-diaminopyrimidines. Growth inhibition tests with *Streptococcus faecium* (ATCC 8043) and antimalarial assays against *Plasmodium berghei* in the mouse and *Plasmodium gallinaceum* in the chick were carried out. One compound, the 6,8-bis(*p*-trifluoromethylphenyl)thiopyranothienopyrimidine analog, was active against *P. berghei* at 640 mg/kg.

In the preceding paper of this series[1] we reported the synthesis of a number of tricyclic 2,4-diaminothieno[2,3-*d*]-pyrimidine ring systems as small-molecule folate antagonists and potential antimalarials. Of interest in connection with the latter aim were two recent patent disclosures concerning the activity shown against chloroquine-resistant *Plasmodium berghei* strains by compounds of general structure **1** (X = MeN, S, SO, or SO_2; Y = Hal, Me, or MeO; Z = basic side chain, *e.g.*, $NHCH_2CH_2NEt_2$).[2-4] In view of these reports it seemed desirable to prepare and test some analogs of **1** in which the typical antimalarial basic side chain has been replaced by 2,4-diamino substitution. The resultant hybrid compounds **2** form the subject of the present paper.



1



2

The overall synthetic plan followed in this work is summarized in Scheme I. The heretofore unknown 1,5-diarylpenta-1,4-dien-3-ones **3b** and **3c** were prepared in high yield according to a standard procedure,[5] starting from 3,4-dichlorobenzaldehyde and 4-trifluoromethylbenzaldehyde, respectively. Further reaction of **3b** with methylamine as reported for the unsubstituted analog **3a** by Lyle and Lyle[6] gave the previously undescribed piperidone **4b** in 61% yield.

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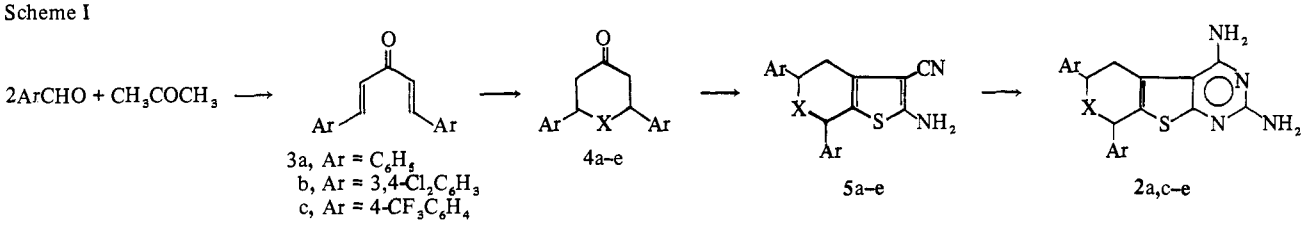
Scheme I



4a, 5a, 2a, X = NMe; Ar = C_6H_5
4b, 5b, X = NMe; Ar = 3,4-$Cl_2C_6H_3$
4c, 5c, 2c, X = S; Ar = C_6H_5
4d, 5d, 2d, X = S; Ar = 3,4-$Cl_2C_6H_3$
4e, 5e, 2e, X = S; Ar = 4-$CF_3C_6H_4$

Similarly, reaction of **3b** and **3c** with H_2S as described for **3a** by Baxter and Whiting[7] afforded high yields of thiacyclohexanones **4d** and **4e**, respectively. It is of interest that whereas condensation of **3b** proceeded normally under the conditions employed with **3a**, a modified procedure had to be developed for **3b** (see Experimental Section). The usual prolonged passage of H_2S gas through a solution of this particular compound resulted solely in the formation of a nonketonic sulfur-containing product whose structure was not elucidated; however, shorter treatment with H_2S led to the desired product in nearly quantitative yield. Condensation of piperidones **4a** and **4b** and of thiacyclohexanones **4c–e** with malononitrile and sulfur in the presence of morpholine or diethylamine, following the general procedure of Gewald and coworkers,[8] led to the formation of the expected aminonitriles **5a–e**. Yields were somewhat lower for the piperidino derivatives **5a** and **5b** than for the thiopyrano derivatives **5c–e** (Table I), perhaps as a consequence of the difference in magnitude between sulfur and nitrogen inductive effects.

Fusion of the aminonitriles with chloroformamidine hydrochloride[9] was performed as described in the preceding paper.[1] The physical constants of the products are shown in Table I. Significantly higher yields and cleaner products were obtained in the condensations of aminonitriles **5c–e** than in those of **5a** or **5b**. The reason for the poor reactivity of nitrogen analogs **5a** and **5b** may be that the basic piperidine nitrogen in these compounds removes HCl from chloroformamidine hydrochloride and interferes with protonation of the aminonitrile moiety, which is necessary to catalyze the formation of the 2,4-diaminopyrimidine ring.

The reaction of 1,5-diphenylpenta-1,4-dien-3-one with methylamine has been shown to give **4a** with the thermodynamically more stable diequatorial *cis*-2,6-diphenyl configuration.[6] Although consideration of steric effects in the transition state for cyclization militates in favor of the trans isomer as the kinetically favored product, it has been suggested[6] that equilibration to the more stable cis isomer can occur *via* a reversal of the Michael addition. In the present work only a single isomer of **4b** was isolated, to which the *cis*-2,6-diaryl configuration was assigned by analogy with the earlier study with **4a**. The cis configuration is likewise assigned to aminonitriles **5a,b** and to **2a**, on the ground that there is no particular reason to expect racemization in the reactions leading to these compounds.

In contrast to the reaction with methylamine, addition of H_2S to 1,5-diphenylpenta-1,4-dien-3-one has been reported to furnish **4c** as a mixture of cis and trans isomers, the former being thermodynamically more stable and hence more abundant at equilibrium.[7] In the present work, the purified cis isomer of **4c** was employed in the synthesis of aminonitrile **5c**; the latter compound is therefore assumed to have its phenyl groups in the cis configuration, as is the final product **2c** derived from **5c** by reaction with chloroformamidine hydrochloride. With thiacyclohexanones **4d,e**, on the other hand, since no special effort was made to separate the 2,6-diaryl isomers on a preparative scale, the aminonitriles **5d,e** and final products **2d,e** are assumed to be predominantly in the cis form.

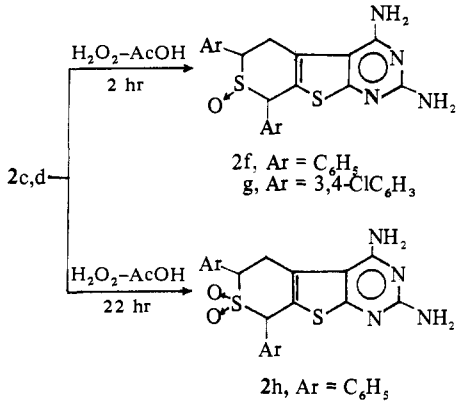
Oxidation experiments were carried out with thiopyran derivatives **2c** and **2d** in order to evaluate the possible

Table I. Physical Constants and Biological Activity of Thieno[2,3-*d*]pyrimidines and Intermediates

Compd	Yield, %	Mp, °C[a]	Crystn solvent	Formula[b]	*S. faecium* inhibition ID_{50}, μg/ml[c]	*P. berghei* in mouse assay Dose, mg/kg	*P. berghei* in mouse assay *T/C*, days
2a	12	267–276 dec	*i*-PrOH[d]	$C_{22}H_{21}N_5S \cdot H_2O$	10[+]	640	6.2/6.1
2c	42	144–150 dec	*i*-PrOH[d]	$C_{21}H_{18}N_4S$	0.1[+]	640	6.4/6.1
2d	31	236–240 dec[e]	EtOH	$C_{21}H_{14}Cl_4N_4S_2$	1.0[+]	640	10.0/6.1
2e	42	243–248 dec	MeCN[f]	$C_{23}H_{16}F_6N_4S_2$	1.0[+]	640	15.6/6.1[g]
2f	91	222–224	THF	$C_{21}H_{18}N_4OS_2$	10[+]	640	10.6/6.1
2g	80	260–264	THF	$C_{21}H_{18}N_4O_2S_2$	10[+]	640	6.6/6.1
2h	80	200–205 dec	MeCN	$C_{21}H_{14}Cl_4N_4OS_2$	1.0[+]	320	6.6/6.1
5a	51	223–224 dec	EtOH	$C_{21}H_{19}N_3S$			
5b	58	260–261 dec	$CHCl_3$–hexane	$C_{21}H_{15}Cl_4N_3S$			
5c	83	228–230 dec	EtOH	$C_{20}H_{16}N_2S_2$			
5d	85	212–214 dec	C_6H_6–hexane	$C_{20}H_{12}Cl_4N_2S_2$			
5e	98	170–178 dec	C_6H_6–hexane	$C_{22}H_{14}F_6N_2S_2$			

[a] Analytical sample (see text for discussion of cis/trans isomers). [b] All compounds were analyzed for C, H, N, and S and, where applicable, Cl or F; compound **2h** was not analyzed for S. [c] Folate concentration = 0.001 μg/ml. [d] Previously chromatographed on silica gel with 10% MeOH–$CHCl_3$ as eluent. [e] Softening at 164°. [f] Previously chromatographed on silica gel with 5% MeOH–$CHCl_3$ as eluent. [g] Compounds producing a 100% or greater increase in survival are defined as active in this test.

Scheme II



effect of increased polarity on the biological activity of these compounds. As indicated in Scheme II, conditions could be devised to effect selective conversion of sulfide **2c** into sulfoxide **2f** or sulfone **2g** in satisfactory yield; similarly, **2d** could be oxidized to sulfoxide **2h**, albeit only in low yield. That oxidation had occurred exclusively on the thiopyran rather than the thiophene or pyrimidine ring was established in each instance by the appearance in the ir spectrum of characteristic sulfoxide or sulfone bands (see Experimental Section). In the nmr spectrum of **2c** (DMSO-d_6 solution), the C-6 and C-8 protons can be seen as multiplets at τ 4.7 and 4.4, respectively. In the spectrum of the sulfoxide **2f**, the C-8 proton appears to be shifted downfield into the NH_2 region (τ 4.0) and the C-6 proton now absorbs at τ 4.4. In the sulfone **2g** even the C-6 proton is shifted into the NH_2 region where it cannot be detected.

Biological Activity. Compounds **2a** and **2c–h** were assayed for antibacterial activity against *Streptococcus faecium* (ATCC 8043) as previously described.[10] As indicated in Table I significant growth inhibition was observed only with **2c**, and even this level of activity was lower by an order of magnitude than that of several simpler 2,4-diaminothieno[2,3-*d*]pyrimidines reported in the accompanying papers of this series.[1,11] Increasing the polar character of the compound by oxidation to sulfoxide **2f** or sulfone **2g** had a markedly unfavorable effect, confirming our earlier impression that activity in this system is promoted by hydrophobic rather than hydrophilic substitution.

Antimalarial testing was likewise performed on these compounds according to the previously described *P. berghei* mouse and *P. gallinaceum* chick assays.[12,‡] Against *P. berghei* (Table I), compound **2c**, which inhibited the growth of *S. faecium*, was devoid of activity. On the other hand, compound **2e**, with CF_3 substitution, caused a 100% increase in mean survival time at 640 mg/kg and is therefore classified as active at this dose. 3,4-Dichloro substitution, as in **2d**, also brought about some increase in the *T/C* value but was less effective than CF_3 substitution. None of the compounds exhibited any activity against *P. gallinaceum*.

On the basis of the available bioassay data, we do not propose further investigation of these variants of the 2,4-diaminothieno[2,3-*d*]pyrimidine ring system.

‡ In the *P. berghei* assay, ICR/Ha mice were infected by intraperitoneal injection of parasitized blood and were given a single subcutaneous dose of compound in oil 3 days after infection. In the *P. gallinaceum* assay, Leghorn chicks were given a single subcutaneous dose in oil immediately after infection *via* the intravenous route.

Experimental Section §

1,5-Bis(3',4'-dichlorophenyl)penta-1,4-dien-3-one (3b). Following the procedure described for dibenzalacetone,[5] 3,4-dichlorobenzaldehyde (175 g, 1 mol) and acetone (29 g, 0.5 mol) were stirred for 1 hr at 20–25° in a solution of NaOH (100 g, 2.5 mol) in 50% EtOH (2 l.): yield 162 g (87%); mp 201–202° (EtOAc). *Anal.* ($C_{17}H_{10}Cl_4O$) C, H, Cl.

Addition of **3b** (11 g, 0.03 mol) and aminoguanidine carbonate (4 g, 0.03 mol) to a mixture of concentrated HCl (4 ml), H_2O (50 ml), and EtOH (250 ml), followed by stirring under reflux for 19 hr, gave the guanylhydrazone·HCl derivative: yield 11 g (81%); mp 262–264° dec (purified by digestion with hot THF). *Anal.* ($C_{18}H_{15}Cl_5N_4$) C, H, Cl, N.

1,5-Bis(4'-trifluoromethylphenyl)penta-1,4-dien-3-one (3c). Use of the foregoing procedure with 4-trifluoromethylbenzaldehyde[#] was found to give a mixture of products, and the following modification was therefore developed. Acetone (2.9 g, 0.05 mol) and 4-trifluoromethylbenzaldehyde (17 g, 0.1 mol) were added to a solution of K_2CO_3 (14 g, 0.1 mol) in 45% EtOH (180 ml), and the mixture was stirred at room temperature for 2 hr: yield 17 g (92%); long needles; mp 156–157° (EtOAc–hexane). *Anal.* ($C_{19}H_{12}F_6O$) C, H, F.

Heating **3c** (3.7 g, 0.01 mol) and aminoguanidine carbonate (1.4 g, 0.01 mol) in $EtOCH_2CH_2OH$ (30 ml) containing concentrated HCl (1.25 ml) for 3 hr under reflux yielded 2.0 g (43%) of guanylhydrazone·HCl derivative, double mp 150–155 and 226–228° dec (purified by digestion with boiling *i*-Pr_2O). *Anal.* ($C_{20}H_{17}ClF_6N_4$) C, H, Cl, F, N.

2,6-Bis(3',4'-dichlorophenyl)-1-methyl-4-piperidone (4b). Gaseous $MeNH_2$ was passed into a warm stirred mixture of **3b** (19 g, 0.05 mol) in MeOH (500 ml) and THF (300 ml) until 15 g of amine had been absorbed. After 7 days at room temperature in a closed flask the orange solution was evaporated to an oil. Chromatography on silica gel (150 g) with benzene as the eluent gave a dark yellow oil which crystallized on cooling and trituration with *i*-Pr_2O (75 ml): yield 12 g (61%); mp 142–143° dec (*i*-Pr_2O, twice). *Anal.* ($C_{18}H_{15}Cl_4NO$) C, H, Cl, N.

2,6-Bis(3',4'-dichlorophenyl)-2,3,5,6-tetrahydrothiopyran-4-one (4d). Gaseous H_2S was bubbled rapidly through a well-stirred suspension of **3b** (5 g, 0.013 mol) and NaOAc (3.7 g, 0.04 mol) in 90% EtOH (150 ml) under reflux. After 5 hr the reaction mixture was poured into H_2O (200 ml) and extracted with Et_2O–THF (1:5 v/v). The organic layer was dried and evaporated, and the residue was triturated with *i*-Pr_2O until crystallization occurred, yield 3.9 g (71%). The crude product, mp 170–174°, was used directly in the next step. The analytical sample was prepared by recrystallization from EtOAc–hexane (1:4 v/v), mp 181–183.5°. *Anal.* ($C_{17}H_{12}Cl_4OS$) C, H, Cl, S.

2,6-Bis(4'-trifluoromethylphenyl)-2,3,5,6-tetrahydrothiopyran-4-one (4e). Application of the foregoing procedure using **3c** instead of **3b** led to the isolation, in high yield, of a compound containing sulfur but lacking carbonyl or olefin absorption (ir, nmr). Accordingly, the following modification was developed. Gaseous H_2S was bubbled rapidly through a well-stirred suspension of **3c** (3.7 g, 0.01 mol) and NaOAc (2.5 g, 0.03 mol) in 90% EtOH (70 ml) under reflux. After only 20 min, the passage of H_2S was stopped and refluxing was continued for another 40 min. The reaction mixture was diluted with H_2O (30 ml) and filtered, yield 3.9 g (97%). This crude product, mp 152–158°, was used directly in the next step. Analytically pure material was obtained by recrystallization from EtOH, mp 169–170°. *Anal.* ($C_{19}H_{14}F_6OS$) C, H, F, S.

2-Amino-5,7-diaryl-3-cyano-4,5,6,7-tetrahydro-6-methylthieno[2,3-*c*]pyridines and 2-Amino-5,7-diaryl-3-cyano-4,5-dihydro-7*H*-thiopyrano[3,4-*b*]thiophenes (5a–e, Table I). Procedure 1. A mix-

§ Uv spectra were measured with Cary Model 11 and Model 15 spectrophotometers. Ir spectra were taken in KCl disks with a Perkin-Elmer Model 137B double-beam recording spectrophotometer. Nmr spectra were determined by means of a Varian A-60 instrument, with Me_4Si as the internal reference. Melting points were measured in Pyrex capillary tubes in a modified Wagner-Meyer apparatus[13] or by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are uncorrected. Microanalyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn., and by Werby Laboratories, Boston, Mass., and are within ±0.4% of theory except where indicated.

We are indebted to Dr. Edgar A. Steck, Walter Reed Army Institute of Research, for furnishing us with a generous sample of this compound.

ture of 4a (15.8 g, 0.06 mol), malononitrile (3.96 g, 0.06 mol), and powdered S (1.92 g, 0.06 g-atom) in 95% EtOH (70 ml) was treated dropwise with morpholine (4.5 ml), warmed to 50° (internal temperature) for 30 min, and stored in the cold until 5a crystallized out: yield 10.5 g (51%); mp 223–224° dec (95% EtOH, twice); ir (KCl) 3550, 3400, 3200, 2210 (C≡N), 1640, 1610 cm^{-1}.

Procedure 2. A well-stirred mixture of 4e (15.8 g, 0.039 mol), malononitrile (2.58 g, 0.039 mol), and powdered S (1.25 g, 0.039 g-atom) in 95% EtOH (100 ml) was treated dropwise with Et_2NH (4 ml) at 40°. When addition was complete the mixture was warmed to 55–60° (internal temperature) for 10 min, cooled, and poured into 0.1 *N* HCl (400 ml), yield 18.5 g (98%). This crude product, mp 95–110° dec, was used directly in the next step. Analytically pure 5e, mp 170–178° dec, was obtained after several recrystallizations from benzene–hexane: ir (KCl) 3400, 3200, 2200 (C≡N), 1600 cm^{-1}.

2,4-Diamino-6,8-diaryl-5,6,7,8-tetrahydro-7-methylpyrido[4',3':4,5]thieno[2,3-*d*]pyrimidines and 2,4-Diamino-6,8-diaryl-5,6-dihydro-8*H*-thiopyrano[4',3':4,5]thieno[2,3-*d*]pyrimidines (2a–e, Table I). Procedure 1. A finely ground mixture of 5a (8.6 g, 0.025 mol) and chloroformamidine hydrochloride (8.6 g, 0.075 mol) was heated in an open pear-shaped flask by means of an oil bath kept at 175°. After 30 min at 175° (internal temperature) the mixture was cooled, transferred to a mortar, pulverized, and digested with hot 0.2 *N* HCl (2 × 600 ml). The digest was decolorized with charcoal, basified with concentrated NaOH, cooled, and filtered. The solid was washed with H_2O, dried, and chromatographed on silica gel (150 g). The fractions eluted with 10% MeOH–$CHCl_3$ (v/v) were combined and recrystallized from *i*-PrOH, yielding 1.3 g (12%) of 2a: almost colorless needles; mp 267–276° dec; ir (KCl) 3400, 1600, 1560, 1530, 1480, 1450 cm^{-1}.

Procedure 2. A mixture of 5e (9.1 g, 0.019 mol) and chloroformamidine hydrochloride (9.1 g, 0.079 mol) was heated as in the preceding experiment. After 20 min at 180° (internal temperature) the melt was cooled, transferred to a mortar, pulverized, digested for 20 min with warm 0.5 *N* NaOH (300 ml), filtered, washed with H_2O, and dried. Chromatography of the tan solid (9.4 g) on silica gel (250 ml) with 5% MeOH–$CHCl_3$ (v/v) as the eluent yielded, after removal of the first few dark-colored fractions, 4.1 g (42%) of 2e as a cream-colored solid: mp 243–248° dec (MeCN); ir (KCl) 3400, 1610, 1550, 1520, 1430, 1410 cm^{-1}.

Oxidation of 2,4-Diamino-6,8-diaryl-5,6-dihydro-8*H*-thiopyrano[4',3':4,5]thieno[2,3-*d*]pyrimidines (2f–h, Table I). Procedure 1. A suspension of 2c (3.5 g, 0.0089 mol) in glacial AcOH (75 ml) and 30% H_2O_2 (23 ml, 0.2 mol) was stirred at room temperature for 1.25 hr, poured into cold H_2O (150 ml), basified with concentrated NH_4OH, and filtered. Pure 2f was obtained by boiling the solid with THF (40 ml): yield 3.3 g (91%); mp 222–224°; ir (KCl) 3450, 3200, 1610, 1550, 1520, 1480, 1440, 1040 cm^{-1} (broad, SO).

Procedure 2. A stirred mixture of 2c (0.5 g, 0.001 mol), glacial AcOH (6 ml), and 30% H_2O_2 (3.2 ml) was kept at room temperature for 22 hr, poured into ice-cold dilute NaOH, and filtered. The yield of 2h was 0.4 g (80%): mp 260–264° (THF); ir (KCl) 3400, 1620, 1590 (broad), 1550, 1520, 1480, 1450, 1320 (SO_2), 1130 cm^{-1} (SO_2).

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2,4-Diaminothieno[2,3-*d*]pyrimidines as Antifolates and Antimalarials. 3. Synthesis of 5,6-Disubstituted Derivatives and Related Tetracyclic Analogs†

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A series of 15 2,4-diaminothieno[2,3-*d*]pyrimidines bearing alkyl, aralkyl, and aryl substituents at the 5 and/or 6 positions was synthesized from the corresponding 2-amino-3-cyanothiophenes and chloroformamidine hydrochloride. Growth inhibition studies with *Streptococcus faecium* (ATCC 8043) revealed significant activity among the 5-alkyl-6-phenyl(or benzyl) derivatives but not the isomeric 6-alkyl-5-phenyl(or benzyl) analogs. Activity was not enhanced by bridging or halogen substituents. The 5-methyl-6-phenyl derivative was active against *Plasmodium berghei* in the mouse at 640 mg/kg, but none of the compounds were active against *P. gallinaceum* in chicks.

A number of 2,4-diamino-5,6,7,8-tetrahydrothianaphtheno[2,3-*d*]pyrimidines and related tricyclic compounds were synthesized in our laboratory as inhibitors of dihydrofolate reductase and as candidate antimalarials.[1,2] Although several of these compounds displayed encouraging activity levels against the folate-requiring microorganism *Streptococcus faecium* (ATCC 8043), their antimalarial activity proved to be at best only marginal. Replacement of a ring carbon in the 5,6-cycloalkano moiety by nitrogen or sulfur resulted in even lower activity, and it was therefore concluded that hydrophobic binding must play a significant role in the formation of a strong

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