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2,4-Diaminothieno[2,3-*d*]pyrimidines as Antifolates and Antimalarials. 1. Synthesis of 2,4-Diamino-5,6,7,8-tetrahydrothianaphtheno[2,3-*d*]pyrimidines and Related Compounds†

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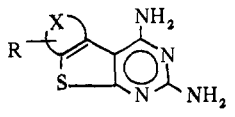
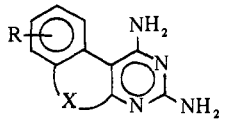
A series of new 2,4-diaminothieno[2,3-*d*]pyrimidine derivatives with a carbocyclic or heterocyclic ring fused at the 5,6 position was synthesized for evaluation as small-molecule folate antagonists. A convenient route to these compounds was condensation of the corresponding 2-amino-3-cyanothiophenes with chloroformamidine hydrochloride, either under dry fusion conditions or with dimethyl sulfone as a diluent. Several of the products inhibited the growth of *Streptococcus faecium* (ATCC 8043) at 0.001–0.01 μg/ml and showed some activity against *Plasmodium berghei* in the mouse and *Plasmodium gallinaceum* in the chick at high doses.

As part of an ongoing survey of condensed 2,4-diaminopyrimidines as small-molecule folate antagonists and potential antimalarial and experimental antitumor agents (for an overall review of our previous work, see ref 1), it was pertinent to synthesize and test some tricyclic 2,4-diaminothieno[2,3-*d*]pyrimidines I. Interest in these compounds was prompted in part by their structural similarity to the several types of tricyclic pyrimethamine analogs II previously synthesized in our laboratory.[2-6] Although considerable work has been done on the chemistry of various other classes of thieno[2,3-*d*]pyrimidines (see, for example, ref 7), systematic investigations of the 2,4-diamino derivatives, which can be viewed as bioisosteres of the 2,4-diaminoquinazolines,[8,9] 2,4-diaminopyrido[2,3-*d*]pyrimidines,[10] and 2,4-diaminopteridines,[11,12] have been sparse. One example, 2,4-diamino-6-benzyl-5-methylthieno[2,3-*d*]-pyrimidine, is reported to be highly active against *Lactobacillus casei* and to exhibit a potentially advantageous selectivity of binding to isolated dihydrofolate reductases of bacterial (*Escherichia coli*) and mammalian (rat liver) origin.[13] The interesting biological properties of this compound provided encouragement for a more extensive synthetic effort, the results of which are reported in this and the accompanying papers.[14,15] Earlier chemical and biological studies on 2,4-diaminothieno[2,3-*d*]pyrimidines conducted independently by Elslager and coworkers as part of a program involving heteroanalogs of the 2,4-diaminoquinazoline antifolates have been reported elsewhere.[16]



I



II

The direct one-step base-catalyzed condensation of ketones with malononitrile and sulfur described by Gewald and coworkers[17] served as the basis for the synthesis of a homologous series of 2-amino-3-cyano-4,5-cycloalkanothiophenes **1a–d** (Table I), starting from cyclohexanone, cycloheptanone, cyclooctanone, and cyclododecanone, respectively. Similarly, 3-methyl-, 4-methyl-, 3,5-dimethyl-, and 4-*tert*-butylcyclohexanone were converted into the corresponding aminonitriles **1e–i**, and the aza analogs **1j–l** were obtained from 1-methyl-, 1-*n*-butyl-, and 1-benzyl-4-piperidone. Aminonitrile **1l** was of interest[16,‡] because of a recent report concerning the analgesic, antipyretic, anti-inflammatory, and platelet aggregation inhibiting activity of the 2-amino-6-benzyl-3-carbethoxy derivative.[19]

The reaction of 3-methylcyclohexanone with malononitrile and sulfur appeared to give a mixture of the two possible products **1e** and **1f**; however, the sterically favored isomer **1e** was assumed to be preponderant. The nmr spectrum of the isomer mixture showed an intense doublet at τ 8.95 (J = 5.5 Hz) attributable to the CHMe grouping in **1e** and a much smaller second doublet slightly downfield at τ 8.82 (J = 5.5 Hz). The latter probably represents a minor amount (less than 10%) of isomer **1f**, in which the Me group would be expected to suffer some deshielding by the neighboring thiophene ring.

The product derived from 3,5-dimethylcyclohexanone

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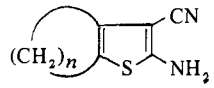
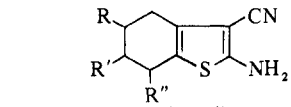
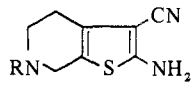
‡Reference to aminonitrile 1l has also been made in a patent.[18]



1a, n = 4
b, n = 5
c, n = 6
d, n = 10



1e, R = Me; R′ = R″ = H
f, R = R′ = H; R″ = Me
g, R = R″ = H; R′ = Me
h, R = R″ = Me; R′ = H
i, R = R″ = H; R′ = *tert*-Bu

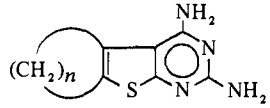


1j, R = Me
k, R = *n*-Bu
l, R = $PhCH_2$

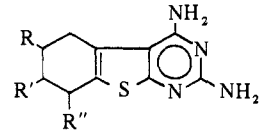
(available commercially as a cis/trans mixture) was assumed to contain both *cis*-**1h** and *trans*-**1h**, the likelihood of stereochemical equilibration to a single isomer being extremely small under the conditions of the reaction.

It is of interest that, whereas the yield of **1a** exceeded 90% and alkyl substitution was without significant effect, appreciably lower yields (40–65%) were observed with cyclic ketones of more than six members. This trend is suggestive of increasing nonbonded repulsive interaction between methylene protons in middle- and large-sized rings fused to a planar five-membered ring.

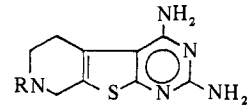
Condensation of the aminonitriles with chloroformamidine hydrochloride[20] at elevated temperatures in the absence of solvent afforded moderate to excellent yields of the desired 2,4-diaminothieno[2,3-*d*]pyrimidines (Table I).§ In a typical run, heating of the fusion components in an open flask led to melting and frothing (HCl gas evolution), followed by gradual resolidification. Melting temperatures varied considerably and not always predictably; for example, in the reaction of **1a** melting occurred at 130°, whereas for **1h** melting was observed at 75° (despite the fact that the melting point of **1h** is 50° higher than that of **1a**). In some reactions which became vigorously exothermic it was necessary to remove the flask from the heating bath. In most instances satisfactory results were obtained by maintaining an internal temperature of 160–185° for 25–45 min.



2a, n = 4
b, n = 5
c, n = 6
d, n = 10



2e, R = Me; R′ = R″ = H
f, R = R′ = H; R″ = Me
g, R = R″ = H; R′ = Me
h, R = R″ = Me; R′ = H
i, R = R″ = H; R′ = *tert*-Bu



2j, R = Me
k, R = *n*-Bu
l, R = $PhCH_2$

In the reaction of **1j**, which failed to give a good melt below 200° (the temperature at which melamine formation becomes an objectionable side reaction), it was found that addition of dimethyl sulfone (mp 109°) as a diluent produced a homogeneous melt at 135°. This technique represents a potentially useful modification of the previously described condensation of aminonitriles with chloroformamidine hydrochloride or cyanoguanidine dihydrochloride.[22]

§ Compounds **2a** and **2l** as well as their aminonitrile precursors **1a** and **1l** were prepared independently by Elslager and coworkers.[16] For an earlier synthesis of **2a**, see ref 21.

Table I. 2,4-Diaminothieno[2,3-*d*]pyrimidines

Compd	Yield, %	Mp, °C	Formula[a]
1b	44	126–127	$C_{10}H_{12}N_2S$
1c	64	98–102	$C_{11}H_{14}N_2S$
1d	42	107–109	$C_{15}H_{22}N_2S$
1e[b]	90	135–138	$C_{10}H_{12}N_2S$
1g	86	140–142	$C_{10}H_{12}N_2S$
1h[c]	80	137–140	$C_{11}H_{14}N_2S$
1i	79	185–187	$C_{13}H_{18}N_2S$
1j	62	192–195	$C_9H_{11}N_3S$
1k	43	149–150	$C_{12}H_{17}N_3S$
1l[d]	71	147–150	$C_{15}H_{15}N_3S$
2a[d]	49	241.5–244	$C_{10}H_{12}N_4S$
2b	76	197–198	$C_{11}H_{14}N_4S$
2c	73	208–210	$C_{12}H_{16}N_4S$
2d	33	231–232.5	$C_{16}H_{24}N_4S$
2e[e]	74	229–233	$C_{11}H_{14}N_4S$
2g	58	251–253	$C_{11}H_{14}N_4S$
2h[f]	73	292–295	$C_{12}H_{16}N_4S$
2i	84	282–284	$C_{14}H_{20}N_4S$
2j	49	192–195	$C_{10}H_{13}N_5S$
2k	92	213–215	$C_{13}H_{19}N_5S$
2l[d,g]	28	189–191.5	$C_{16}H_{17}N_5S$

[a] All compounds were analyzed for C, H, N, and S. [b] Probably contains some **1f** (R = R′ = H; R″ = Me). [c] *cis*/*trans*-5,7-Me_2 mixture. [d] Synthesized independently by Elslager, *et al.*[16,‡] [e] Probably contains some **2f** (R = R′ = H; R″ = Me). [f] *cis*/*trans*-6,8-Me_2 mixture. [g] Methiodide salt, mp 182–199° dec (MeOH).

2,4-Diaminothieno[2,3-*d*]pyrimidine derivatives **2a–l** were characterized in part on the basis of ultraviolet absorption spectra. A typical spectrum, that of compound **2h**, showed maxima at 238 and 277 nm in EtOH and at 244 and 272.5 nm in 0.1 *N* HCl. The concurrent bathochromic and hypsochromic shifts shown in acid solution by the low- and high-wavelength bands, respectively, of these compounds are noteworthy. In 2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidine both bands undergo bathochromic shifts in acid solution,[23] whereas in 2,4-diaminoquinazoline they both exhibit hypsochromic shifts.[24] Thus, 2,4-diaminothieno[2,3-*d*]pyrimidines may perhaps be viewed as intermediate, electronically, between these two ring systems, in accord with the concept that thiophenes are less π excessive than pyrroles.[25]

Biological Activity. Compounds **2a–l** were assayed for growth-inhibitory activity against *Streptococcus faecium* (ATCC 8043) as previously described.[26] The results (Table II) suggest that inhibition is promoted by hydrophobic substitution at the 5 and 6 positions of the 2,4-diaminothieno-[2,3-*d*]pyrimidine ring system. The introduction of alkyl substituents on the cyclohexane ring results in a marked enhancement of activity (compare **2e–i** with **2a**). In the most favorable instances ID_{50} values in the 0.001–0.01 μg/ml range can be obtained which are comparable to those reported for the most active tricyclic pyrimethamine analogs.[1] Expansion of the cycloalkane ring to more than six members likewise affects the activity, albeit to a lesser degree. The seven-membered analog **2b** is appreciably more active than **2a**, but the eight-membered and twelve-membered analogs **2c** and **2d** are not greatly different. Replacement of a single ring carbon by nitrogen (compare **2g** and **2j**) leads to a greater than tenfold loss of activity and further replacement of the *N*-methyl by more hydrophobic *N*-*n*-butyl or *N*-benzyl groups (compare **2k** and **2l** with **2j**) does not restore activity. Quaternization of the nitrogen (compare **2j** or **2l** with **2l**·MeI) leads to still greater loss of activity.

Table II. Antibacterial and Antimalarial Evaluation of 2,4-Diaminothieno[2,3-d]pyrimidine Derivatives

Compd	*S. faecium* (ATCC 8043)	*P. berghei* in mouse		*P. gallinaceum* in chick	
	ID_{50}, μg/ml[a]	Dose, mg/kg	*T/C*, days	Dose, mg/kg	*T/C*, days
2a	0.15	640	6.2/6.1	40	4.0/4.0
2b	0.015	640	6.6/6.1	100	4.0/4.0
2c	0.26	640	13.2/6.1[b]		
2d	0.06	640	6.2/6.1		
2e,f	0.005	640	12.0/6.1	320	10.8/4.0[b]
2g	0.001	640	9.2/6.1	320	9.8/4.0[b]
2h	0.007	640	10.6/6.1	120	14.0/4.0[b]
2i	0.004	640	7.2/6.1	120	4.0/4.0
2j	1.0+	160[c]	6.4/6.1	320	4.0/4.0
2k	1.0+	640	6.4/6.1	320	4.0/4.0
2l	1.5	160[c]	6.4/6.1	120	4.0/4.0
2l·MeI	3.0	40[d]	6.2/6.1	160	4.0/4.0

[a] Folate concentration = 0.001 μg/ml. [b] Active (*T/C* > 100%). [c] Toxic to 5/5 animals at 640 mg/kg. [d] Toxic to 5/5 animals at 160 mg/kg.

Six compounds (**2a**, **2b**, and **2h-k**) were also tested *in vivo* against two transplantable mouse leukemias in ascitic form: L1210 leukemia in BDF/1 hybrid mice and P1534 leukemia in DBA/2 inbred mice. Compounds were injected intraperitoneally daily for 4 days beginning on the first day after tumor implantation, at several dose levels. At nontoxic doses none of the compounds showed significant activity in either tumor system.

Antimalarial assays were performed against *Plasmodium berghei* in the mouse and *Plasmodium gallinaceum* in the chick according to the published procedure.[27] In the *P. berghei* assay, ICR/Ha mice were injected with a single subcutaneous dose of compound in oil 3 days after intraperitoneal infection. In the *P. gallinaceum* assay, chicks were given a single subcutaneous dose in oil immediately after intravenous infection. The results are summarized in Table II. It can be concluded from the data that hydrophobic substitution at the 5 and 6 positions of the 2,4-diaminothieno-[2,3-*d*]pyrimidine ring system can, in certain instances, produce a slight enhancement in antimalarial activity in both test systems (compare **2e,f-h** with **2a**). However, this is not always true (see **2b-d** and **2i**). Replacement of a ring carbon by nitrogen (compare **2j-l** with **2a**) also fails to produce activity; in fact, this particular modification yields compounds that are markedly toxic to the mouse. As in the *Strep. faecium* assay, alkyl groups on the cyclohexane ring have a favorable effect on activity. Similarly, increasing the size of the cycloalkane ring is beneficial, although this becomes evident only with the eight-membered analog **2c** rather than the seven-membered analog **2b**. In general, even the best compounds in this series exhibit significant activity only at the highest doses and are clearly inferior to previously synthesized tricyclic pyrimethamine types.[1]

Experimental Section#

The following are representative synthetic procedures for the compounds in this study.

2-Amino-3-cyano-4,5,6,7-tetrahydro-6-methylthianaphthene (1g). A well-stirred mixture of 4-methylcyclohexanone (22.4 g, 0.2 mol), malononitrile (13.2 g, 0.2 mol), and sulfur (6.72 g, 0.2 g-atom) in 95% EtOH (110 ml) was treated dropwise with Et_2NH (20 ml), the internal temperature being maintained below 60° by means of an ice bath. When addition was complete, the mixture was stirred 1.5 hr at ambient temperature, then poured into H_2O (450 ml), and filtered. Recrystallization of the solid (36.6 g, 86% yield) from 95% EtOH (charcoal) gave colorless needles.

2-Amino-6-*n*-butyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]-pyridine (1k). A well-stirred mixture of 1-*n*-butyl-4-piperidone (54 g, 0.35 mol), malononitrile (23 g, 0.35 mol), and sulfur (13 g, 0.4 g-atom) in 95% EtOH (220 ml) was cooled in an ice bath and treated dropwise with Et_2NH (35 ml). When addition was complete the reaction mixture was warmed to 60° (internal) for 15 min and then stored in the cold until crystallization occurred. The product (36 g, 43% yield) was recrystallized from EtOAc–hexane.

2,4-Diamino-5,6,7,8-tetrahydro-6,8-dimethylthianaphtheno-[2,3-*d*]pyrimidine (Cis/Trans Mixture) (2h). A finely ground mixture of **1h** (10 g, 0.048 mol) and chloroformamidine hydrochloride[20] (10 g, 0.087 mol) was heated in an open pear-shaped flask by means of an oil bath. The internal temperature was raised to 140° over a 20-min period and maintained at 140–160° for an additional 25 min. Melting occurred above 75° and foaming was evident above 135°, with gradual solidification of the melt toward the end of the reaction. The cooled product was pulverized in a mortar, triturated with 0.1 *N* NaOH (1200 ml), filtered, washed with H_2O, and digested with hot 1 *N* HCl (800 ml). A small amount of gummy residue was removed and the hot filtrate** was decolorized with charcoal and basified with 10% NaOH (300 ml). The resulting solid (8.8 g, 73% yield) was recrystallized from 95% EtOH (charcoal).

2,4-Diamino-7-*tert*-butyl-5,6,7,8-tetrahydrothianaphtheno[2,3-*d*]pyrimidine (2i). A finely ground mixture of **1i** (15 g, 0.064 mol) and chloroformamidine hydrochloride (15 g, 0.13 mol) was heated for 30 min in an oil bath maintained at 180° (bath temperature). The cooled melt was pulverized in a mortar, triturated for 20 min with 0.5 *N* NaOH (300 ml), filtered, washed with H_2O, and recrystallized from EtOH containing some Et_2NH to assist solubilization, yield 15 g (84%).

2,4-Diamino-5,6,7,8-tetrahydro-7-methylpyrido[4′,3′:4,5]thieno-[2,3-*d*]pyrimidine (2j). A finely ground mixture of **1j** (10 g, 0.052 mol), chloroformamidine hydrochloride (10 g, 0.087 mol), and dimethyl sulfone (50 g) was heated for 30 min in an oil bath kept at 160° (bath temperature). A homogeneous melt was observed at 135° and the internal temperature rose exothermally to a maximum of 174° before subsiding gradually. The crude resolidified melt was worked up as in the preceding experiment and recrystallized from absolute EtOH (1100 ml) (charcoal), yield 6 g (49%).

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#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 tetramethylsilane as the internal reference. Melting points were measured in Pyrex capillary tubes in a modified Wagner-Meyer apparatus[28] 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 Werby Laboratories, Boston, Mass., and are within ±0.5% of theory except where otherwise noted.

**It is advisable to carry out filtration of the acid digest while it is hot, in order to avoid crystallization of the hydrochloride which sometimes occurs.

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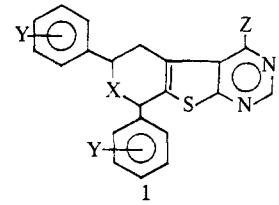
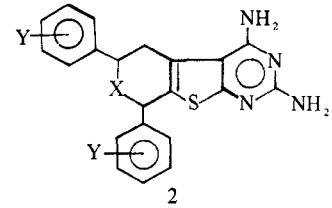
Several new 2,4-diamino-6,8-diaryl-5,6-dihydro-8*H*-thiopyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidines, including some sulfoxide and sulfone derivatives, were synthesized as candidate antifolate antimalarials. One example of the 2,4-diamino-6,8-diaryl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidine 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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