Thiazolo[4,5-*d*]pyrimidines. Part I. Synthesis and Anti-Human Cytomegalovirus (HCMV) Activity *in vitro* of Certain Alkyl Derivatives

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Alkyl derivatives of the thiazolo[4,5-*d*]pyrimidine congeners of guanine and uracil were prepared and assessed for *in vitro* activity against human cytomegalovirus (HCMV). The finding that the 3-pentyl **1b** and 3-hexyl **1c** derivatives of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1e**) had potent *in vitro* anti-HCMV activity prompted a broader study of alkyl derivatives in this ring system. A series of 3-alkyl derivatives of **1e**, *viz.* **1f-w**, were prepared by direct alkylation of the sodium salt of **1e** and by subsequent modifications, **2a-d**. For comparison with **1c**, 5-amino-2-hexylaminothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**4**) was prepared and studied. The 3-(2-alkenyl) derivatives of **1e** were found to be the more active antiviral agents with the *Z* isomer of 5-amino-3-(2-penten-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1f**) having the better therapeutic index. Analogous 4-(2-alkenyl) derivatives of 2-aminothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione **6a** and **6b** were also prepared but were found to have poor therapeutic indices. Single crystal X-ray diffraction analysis was used to unequivocally establish the structure of **1f**.

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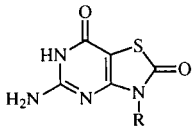
Certain 9-alkylguanines exhibit marked antiviral activity in animal models but are devoid of *in vitro* activity [1]. The *in vivo* antiviral activity of these guanine derivatives has been ascribed [1] to immune potentiation. Similarly, in a separate study [2], the guanosine congener 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1a**) showed only *in vivo* antiviral activity resulting from induction of interferon. We recently described [3] the synthesis of the 3-*n*-pentyl **1b**, 3-*n*-hexyl **1c**, and 3-(6-hydroxy-*n*-hexyl) **1d** derivatives of the guanine analogue 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1e**). These compounds, whose primary mode of action was expected to be potentiation of the host immune system, were subsequently found to have potent *in vitro* activity (see Table 1) against human cytomegalovirus (HCMV). This unexpected finding suggested that these compounds were acting by a mechanism other than immune potentiation. Further, these alkyl guanine analogues should not be phosphorylated; thus, they would not act as viral polymerase inhibitors in a manner similar to the acyclonucleosides such as acyclovir and ganciclovir [4]. This latter suggestion was supported by the inactivity of compounds **1a** and **1d** in an identical cell culture experiment.



1

a, R = β-D-ribofuranosyl
b, R = $(CH_2)_4CH_3$
c, R = $(CH_2)_5CH_3$
d, R = $(CH_2)_5CH_2OH$
e, R = H

In addition to their antiviral activity, **1b** and **1c** were found to be significantly cytotoxic to uninfected cells. Thus, we initiated structure-activity studies with respect to the alkyl thiazolo[4,5-*d*]pyrimidines with the goal of obtaining anti-HCMV agents with improved therapeutic indices. The results of our initial efforts are the subject of this report.

Compounds **1f-w** (Scheme 1) were synthesized by direct alkylation of the sodium salt of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1e**) [5]. Commercially available alkyl halides were used for the synthesis of all of the structure **1** alkyl derivatives with the exception of **1g**, **1m**, **1o**, and **1p**. The temperature and the duration of the reaction were varied to accommodate a difference in the reactivity of the respective alkyl halide, but, with the exception of **1f** and **1h**, the reaction conditions and isolation methodology were not optimized. The site of alkylation was assigned in each case by comparisons of the uv spectra of the new compound with the spectra of the established 3-β-D-ribofuranosyl derivative **1a** [2] and was supported by the single crystal X-ray analysis of **1f**. Compounds **2a** and **2b** were obtained by the treatment of **1s** with sodium hydroxide and ammonium hydroxide, respectively.

Table 1

In Vitro Antiviral (HCMV) Activity and Cytotoxicity of Alkyl Thiazolo[4,5-*d*]pyrimidines

Compound No.	ED_{50}[a]μM	CD_{50}[b]μM	TI [c]
1a	>290 [d]	>290 [d]	
1b	1	15	15
1c	2	6	3
1d	120	>100 [d]	
1f	<0 .4 [e]	51	>127
1g	4	61	15
1h	<4 [e]	>100 [d]	>25
1i	16	56	4
1j	37	270	7
1k	inactive	57	
1l	6	23	4
1m	4	60	15
1n	inactive	130	
1o	30	570	19
1p	36	150	4
1q	6	156	26
1r	170	120	
1t	10	15	1
1u	9	5	
1v	5	10	2
1w	69	49	
2a	>350 [d]	>350 [d]	1
2b	>350 [d]	>350 [d]	1
2c	32	120	4
2d	45	250	6
4	ND [f]	42	
6a	24	27	1
6b	inactive	60	
ganciclovir	5-30 [g]	>1000	

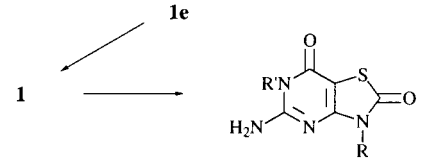
[a] ED_{50} = effective dose at 50% level. [b] CD_{50} = cytotoxic dose at 50% level. The test compounds were evaluated (in quadruplicate) for visual cytotoxicity in stationary uninfected MRC-5 cells using a scoring of 0 (no visual cytotoxicity at 20 fold magnification) to 4 (cell sheet nearly destroyed). Each assay was performed in quadruplicate. The resultant data were averaged, graphed and then used to calculate the CD_{50}. [c] TI = therapeutic index (CD_{50}/ED_{50}). [d] Highest concentration tested. [e] Lowest concentration tested. [f] Not determined. [g] Value range over 19 experiments.

The synthesis of the pentynyl derivative **1m** required the preparation of 1-bromo-2-pentyne which was synthesized from the corresponding alcohol according to the method of Kajiwara *et al.* [6]. The α carbon protons of the alkyne chain appeared as a doublet of doublets in the ^{1}H nmr spectrum of **1m**, indicating a nonequivalence of these protons induced by the heterocyclic base.

To accomplish the synthesis of **1o** and **1p**, 5-bromononane and 6-bromoundecane were prepared by the method described by Katritzky and coworkers [7]. A nonequivalence of the methylene protons on the β carbons was observed in the ^{1}H nmr spectra for the branched chain derivatives **1n**, **1o**, and **1p**.

Treatment of the sodium salt of **1e** with 1-bromo-2-pentene [sold (Aldrich) as the predominately *E* isomer] gave a product which was found, by hplc analysis, to be a 19:1 mixture of geometric isomers. The olefinic coupling constant (^{1}H nmr) of the major isomer was 11 Hz, which did not allow unequivocal assignment of the stereochemistry for this olefin. Treatment of **1e** with (*Z*)-1-bromo-2-pentene, prepared by a known procedure [8], also gave a product with an 11 Hz coupling constant for the olefinic protons and an identical hplc profile to the above product. Single crystal X-ray diffraction analysis of the product obtained from commercial 1-bromo-2-pentene clearly showed the major isomer to be the *Z* olefin; thus, this product was assigned the structure **1f** and, by default, the minor product was assigned structure **1g**. Later analysis confirmed that the commerical 1-bromo-2-pentene was in fact the *Z* geometric isomer. For the synthesis of the *E* olefin, (*E*)-1-bromo-2-pentene [8] was allowed to react with the sodium salt of **1e** to obtain **1g** which was shown by hplc analysis to contain approximately 1% of the *Z* olefin **1f**. The larger olefinic coupling constant (15 Hz) for **1g** confirmed its assignment as the *E* isomer. For biological comparisons the *Z* and the *E* olefin, each containing less than 0.6% of the opposite geometric isomer, were obtained after preparative hplc separation .

Scheme 1



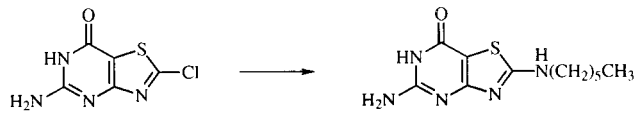
2

f, R = -$CH_2CH{=}CHCH_2CH_3$ (*Z*)
g, R = -$CH_2CH{=}CHCH_2CH_3$ (*E*)
h, R = -$CH_2CH{=}C(CH_3)_2$
i, R = -$(CH_2)_2CH{=}C(CH_3)_2$
j, R = -$(CH_2)_3CH{=}CH_2$
k, R = -2-cyclohexen-1-yl
l, R = -$CH_2CH{=}CHC_6H_5$ (*E*)
m, R = -$CH_2C{\equiv}CCH_2CH_3$
n, R = -$CH(CH_2CH_3)_2$
o, R = -$CH[(CH_2)_3CH_3]_2$
p, R = -$CH[(CH_2)_4CH_3]_2$
q, R = -tetrahydrofurfuryl
r, R = -$(CH_2)_4CN$
s, R = -$(CH_2)_4CO_2C_2H_5$
t, R = -$CH_2C_6H_5$
u, R = -$CH_2C_6H_4$(*p*-Br)
v, R = -$CH_2C_6H_4$(*p*-Cl)
w, R = -$CH_2C_6H_4$(*p*-CN)

a, R = -$(CH_2)_4CO_2H$, R' = H
b, R = -$(CH_2)_4CONH_2$, R' = H
c, R = -$CH_2CH{=}CHCH_2CH_3$ (*Z*), R' = -CH_3
d, R = -$CH_2CH{=}CHCH_2CH_3$ (*Z*), R' = -NH_2

The effect on biological activity of relocating the alkyl chain from the 3 position of the base to an exocyclic amino function at the 2 position was assessed by the synthesis and study of 5-amino-2-hexylaminothiazolo[4,5-*d*]-pyrimidin-7(6*H*)-one (**4**, Scheme 2). Treatment of 5-amino-2-chlorothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**3**) [5] with aqueous hexylamine yielded **4**.

Scheme 2



The *in vitro* antiviral (HCMV) and cytotoxicity data for this series of compounds are collated in Table 1. Antiviral effectiveness for each compound was derived from plaque reduction experiments using the AD169 strain of HCMV in MRC-5 cells and the methodology described by Barnard *et al.* [9]. All compounds were scored for visual cytotoxicity in uninfected, stationary MRC-5 cells. For comparison, a normal value range determined concurrently and in an identical manner is given for ganciclovir.

The 3-pentyl **1b** and 3-hexyl **1c** derivatives of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione showed good activity as antiviral agents but had poor therapeutic indices because of their high cytotoxicity. The data from Table 1 clearly demonstrate that the most effective means of improving the therapeutic index [TI, cytotoxic dose at 50% level (CD_{50})/effective dose at 50% level (ED_{50})] of these 3-alkyl derivatives was to introduce unsaturation into the alkyl chain. The position of the unsaturation was also a critical factor in obtaining maximum efficacy. Thus, the 2-enes, **1f-1h**, demonstrated high activity (low ED_{50}) and low toxicity (high CD_{50}) in cell culture, whereas a 3-ene **1i** or a 4-ene **1j** was comparatively less active, while the shorter, phenyl substituted 2-alkene **1l** again had a small therapeutic index. The degree of unsaturation may not be important since the 2-pentyne derivative **1m** had comparable activity to **1g** and **1h**. However, incorporating the unsaturation into a cyclic moiety, *e.g.* **1k**, resulted in complete loss of activity. Of more significance was the geometry about the olefinic bond; thus, the *Z* olefin **1f** showed superior activity in comparison to the corresponding *E* isomer **1g**. Since the initial antiviral activity was found for compounds with 5 and 6 carbon alkyl chains, this study on the effect of unsaturation was restricted, with the exception of **1l**, to pentyl and hexyl derivatives of **1e**.

The introduction of branched alkyl chains such as in **1n-p** resulted not only in reduced cytotoxicity but also diminution of antiviral activity when compared to **1b** or **1c**. Comparing the results from the three branched derivatives suggests a possible correlation of chain length to both activity and cytotoxicity.

The inclusion of functional groups on the terminus of the alkyl chains resulted in either inactive compounds, **1d**, **2a** and **2b** or reduced efficacy, **1r**. However, the tetrahydrofurfuryl derivative **1q** had a better therapeutic index than all of the compounds tested except **1f** and **1h**.

Replacement of the alkyl chain with aralkyl derivatives **1t-w** produced compounds with very small therapeutic indices. The only modest improvement in cytotoxicity achieved by moving the alkyl chain to an exocyclic 2-amino group discouraged further investigation of 2-alkylaminothiazolo[4,5-*d*]pyrimidines.

Certain tricyclic analogues of acyclovir and ganciclovir have been reported to display significant and selective antiherpetic activity [10]. Additionally, structural analogs [11] of the "Y" base of phenylalanine specific transfer ribonucleic acids ($tRNA^{Phe}$) are of continued interest. Following these leads we sought to prepare similar tricyclic analogues of the active 2-pentenyl compound **1f**. The sodium salt of **1f** (predominantly the *Z* olefin) was treated with iodomethane to obtain **2c** (predominantly the *Z* olefin), whereas, adopting the methodology developed by Broom and Robins [12], treatment with hydroxylamine-*O*-sulfonic acid produced **2d** (predominantly the *Z* olefin). The diminished antiviral activity resulting from substitution of the lactam nitrogen of **1f**, as shown by **2c** and **2d**, precluded further synthetic efforts with respect to the tricyclic derivatives.

Through the past two decades there has been continued interest in the synthesis and biological evaluation of 3-ribosylpurines [13] and analogous nucleosides in other ring systems [14-16]. Therefore, it was of interest to examine thiazolo[4,5-*d*]pyrimidines with alkyl substituents in the pyrimidine ring. Preliminary efforts in this regard led to the synthesis of compounds **6a** and **6b** (Scheme 3) by alkylation of the sodium salt of 2-aminothiazolo[4,5-*d*]-pyrimidine-5,7(4*H*,6*H*)-dione (**5**) [5]. The site of alkylation was assigned from comparisons of the uv spectra of **6a** and **6b** with those for the established 2-amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione [15]. Because of the small therapeutic indices displayed by **6a** and **6b**, the synthesis of other 4-alkylthiazolo[4,5-*d*]-pyrimidine derivatives was not pursued.

Scheme 3

5 **6**

a, R = $-CH_2CH{=}CHCH_2CH_3$ (*Z*)
b, R = $-CH_2CH{=}C(CH_3)_2$

5-Amino-3-[(*Z*)-2-penten-1-yl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1f**) and 5-amino-3-(3-methyl-2-buten-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1h**) were chosen as lead compounds for further evaluation. Of the two, **1f** showed the better spectrum of antiviral activity. This agent displayed *in vitro* activity against other laboratory strains of HCMV, primary isolates of HCMV, ganciclovir resistant strains (both polymerase and kinase mutants) of HCMV, murine CMV (MCMV), herpes simplex virus (HSV) type 1 and type 2, and TK^- (thymidine kinase deficient) HSV-2. However, compound **1f** was unable to protect mice (intraperitoneal administration) against lethal challenges with either HSV or MCMV. These additional studies are detailed in a separate manuscript [17].

The concept of substituting a natural or modified nucleic acid base with an unsaturated alkyl moiety to produce an antiviral agent is not novel; the recent literature is replete

Table 2

Crystal and Experimental Data for Compound **1f**

A. Crystal Data

Empirical formula	$C_{10}H_{12}N_4O_2S$
Formula weight	252.29
Crystal color, habit	yellow, plate
Crystal dimensions (mm)	0.35 x 0.15 x 0.08
Crystal system	orthorhombic
Lattice Type	primitive
No. of reflections used for unit cell determination (2θ range)	25 (46.8 - 49.9°)
Omega scan peak width at half-height	0.19°
Lattice parameters:	a = 12.899 (2) Å b = 20.733 (2) Å c = 4.483 (2) Å V = 1198.8 (5) Å^3
Space group	$Pna2_1$ (#33)
Z value	4
D_{calc}	1.398 g/cm^3
F_{000}	528 (electrons)
μ(CuKα)	23.95 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (λ = 1.54178Å)
Temperature	23°
Take-off angle	6.0°
Detector aperture	9.0 mm horizontal 13.0 mm vertical
Crystal to detector distance	40 cm
Scan type	ω-2θ
Scan rate	16.0°/min (in ω) (up to 4 rescans)
Scan width	(1.37 + 0.35 tan θ)°
$2\theta_{max}$	106.1°
No. of reflections measured	Total: 2253 Unique: 1526
Corrections	Lorentz-polarization Absorption (trans. factors: 0.8535-0.9923) Secondary extinction (coefficient: 6.44761e-06)

C. Structure Solution and Refinement

Structure solution	Direct methods
Refinement	Full-matrix least-squares
Function minimized	$\Sigma\ \omega\ (|Fo| - |Fc|)^2$
Least-squares weights	$1/\sigma^2(Fo) = 4Fo^2/\sigma^2(Fo^2)$
p-factor	0.014
Anomalous dispersion	All non-hydrogen atoms
No. observations [I>3.00σ(I)]	712
No. variables	104
Reflection/parameter ratio	6.85
Residuals R; R_w	0.051; 0.061
Goodness of fit indicator	3.27
Max. shift/error in final cycle	0.00
Maximum peak in final diff. map	0.28 e$^-$/Å^3
Minimum peak in final diff. map	-0.31 e$^-$/Å^3

Table 3

Intramolecular Bond Lengths (Å) for Compound **1f**

atom	atom	distance	atom	atom	distance
S1	C2	1.772 (8)	S1	C8	1.739 (7)
C2O	C2	1.217 (8)	C7O	C7	1.260 (8)
N3	C2	1.357 (10)	N3	C3	1.379 (8)
N3	C1'	1.469 (9)	N4	C3	1.33 (1)
N4	C5	1.342 (7)	C5N	C5	1.343 (10)
N6	C5	1.348 (8)	N6	C7	1.373 (9)
C3	C8	1.360 (9)	C7	C8	1.422 (9)
C1'	C2'	1.48 (1)	C2'	C3'	1.33 (1)
C3'	C4'	1.49 (1)	C4'	C5'	1.42 (1)

Estimated standard deviations in the least significant figure are given in parentheses.

with examples [18-24]. However, with few exceptions these compounds all contain phosphorylatable moieties on the alkyl chain or are phosphonates. The structure of **1f** coupled with its *in vitro* antiviral profile is suggestive of a new class of antiherpetic agent which is exerting its effect through an undetermined mechanism. An understanding of the inability of **1f** to protect animals against lethal viral challenges may require elucidation of its mode of action and further work will be necessary to clarify these issues.

Crystals of **1f** suitable for X-ray diffraction analysis were obtained from a methanol-water solution. The structure was solved by direct methods and expanded using Fourier techniques. Some non-hydrogen atoms were refined anisotropically, while the rest were refined isotropically. Hydrogen atoms were included but not refined. A perspective drawing of **1f** is shown in Figure 1. The crystal and experimental data derived from the X-ray diffraction study are summarized in Table 2, and intramolecular bond lengths, intramolecular bond angles, and torsion angles for compound **1f** are given in Tables 3-5.

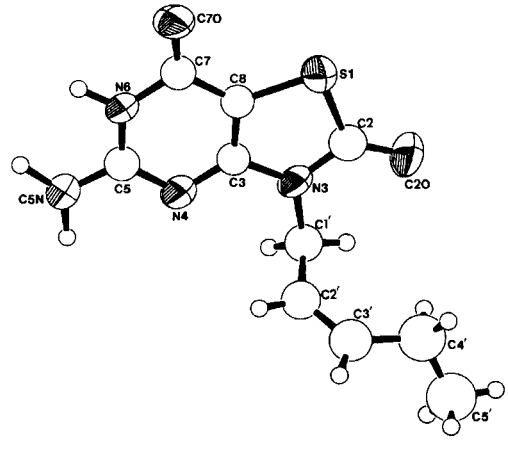


Figure 1. Perspective drawing of compound **1f** illustrating atom labelling and molecular conformation. The thermal elipsoids were determined at the 50% probability level.

Table 4

Intramolecular Bond Angles (°) for Compound **1f**

atom	atom	atom	angle	atom	atom	atom	angle
C2	S1	C8	89.8 (4)	C2	N3	C3	115.4 (6)
C2	N3	C1'	122.2 (6)	C3	N3	C1'	122.4 (6)
C3	N4	C5	113.7 (6)	C5	N6	C7	125.0 (6)
S1	C2	C2O	124.7 (7)	S1	C2	N3	109.9 (5)
C2O	C2	N3	125.3 (7)	N3	C3	N4	120.5 (6)
N3	C3	C8	112.3 (7)	N4	C3	C8	127.2 (6)
N4	C5	C5N	119.6 (7)	N4	C5	N6	122.7 (7)
C5N	C5	N6	117.7 (6)	C7O	C7	N6	120.7 (6)
C7O	C7	C8	126.9 (7)	N6	C7	C8	112.4 (7)
S1	C8	C3	112.6 (5)	S1	C8	C7	128.3 (6)
C3	C8	C7	119.0 (7)	N3	C1'	C2'	111.8 (7)
C1'	C2'	C3'	127.0 (7)	C2'	C3'	C4'	128.3 (8)
C3'	C4'	C5'	117.2 (8)				

Estimated standard deviations in the least significant figure are given in parentheses.

Table 5

Torsion Angles (°) for Compound **1f**

atom	atom	atom	atom	angle
S1	C2	N3	C3	0.7 (9)
S1	C2	N3	C1'	-179.1 (6)
S1	C8	C3	N3	-0.5 (9)
S1	C8	C3	N4	-179.5 (8)
S1	C8	C7	C7O	-1 (1)
S1	C8	C7	N6	180.0 (6)
C2O	C2	S1	C8	-179.1 (8)
C2O	C2	N3	C3	178.9 (9)
C2O	C2	N3	C1'	0 (1)
C7O	C7	N6	C5	-177.8 (8)
C7O	C7	C8	C3	175.6 (8)
N3	C2	S1	C8	-0.8 (6)
N3	C3	N4	C5	-179.1 (7)
N3	C3	C8	C7	-178.3 (8)
N3	C1'	C2'	C3'	-113.8 (10)
N4	C3	N3	C2	178.9 (8)
N4	C3	N3	C1'	-1 (1)
N4	C3	C8	C7	2 (1)
N4	C5	N6	C7	1 (1)
C5N	C5	N4	C3	178.4 (8)
C5N	C5	N6	C7	-178.5 (8)
N6	C5	N4	C3	-2 (1)
N6	C7	C8	C3	-2 (1)
C2	S1	C8	C3	0.8 (6)
C2	S1	C8	C7	178.3 (8)
C2	N3	C3	C8	-0.1 (10)
C2	N3	C1'	C2'	99.9 (9)
C3	N3	C1'	C2'	-80.0 (9)
C5	N4	C3	C8	0 (1)
C5	N6	C7	C8	0 (1)
C8	C3	N3	C1'	179.7 (7)
C1'	C2'	C3'	C4'	2 (1)
C2'	C3'	C4'	C5'	-136 (1)

The sign of the angle is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4. Estimated standard deviations in the least significant figure are given in parentheses.

EXPERIMENTAL

Melting points (mp) were determined with a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. Ultraviolet (uv) spectra (sh = shoulder) were recorded with a Hewlett-Packard 8452 diode array spectrophotometer. Infrared (ir) spectra were recorded in potassium bromide with a Perkin-Elmer 1420 ir spectrophotometer. Nuclear magnetic resonance (^{1}H nmr) spectra were recorded in dimethyl sulfoxide-d_6 with a Bruker AM400 wide bore spectrometer and the chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (internal) (key: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, and br = broad). Single crystal X-ray diffraction analysis was performed by Molecular Structure Corporation, The Woodlands, Texas. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, New Jersey. Thin layer chromatography (tlc) was conducted on aluminum plates coated (0.2 mm) with silica gel 60F_{254} (EM Science) and components were visualized by uv absorbance. EM Science silica gel 60 Å (230-400 mesh) was used for all column chromatographic separations. Analytical high pressure liquid chromatography (hplc) analyses used a Waters system equipped with a model 996 photo diode array (PDA) detector and was performed on a 25 x 4.6 cm Supelcosil LC-18-DB (5μ) column. Preparative hplc used a Waters Delta Prep 3000 equipped with a model 991 PDA detector and the separations were accomplished on a PrepPAK 500 C_{18} column. Evaporations were carried out at a temperature ≤35° and under diminished pressure for solvents with bp <80° or at a temperature ≤50° and under high vacuum for higher boiling solvents. All chemicals used were reagent grade and were not further dried or purified unless otherwise noted.

5-Amino-3-[(*Z*)-2-penten-1-yl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1f**).

A mixture of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione [5] (**1e**, 3.0 g, 16.3 mmoles), sodium hydride (80% dispersion in mineral oil, 0.525 g, 17.5 mmoles), and anhydrous *N,N*-dimethylformamide (60 ml) was protected from moisture and stirred at ambient temperature for 1 hour. (*Z*)-1-Bromo-2-pentene (predominantly the *Z* olefin, 2.4 ml, 20.3 mmoles) was added and the mixture was heated at 75 ± 3° for 4.5 hours. The mixture was evaporated and then water (100 ml) was added. The mixture was stirred for 15 minutes, the solid was collected by filtration, and crystallized from a methanol-water mixture. The compound was dried in vacuum at 100° for 16 hours; 2.93 g (11.5 mmoles, 71%), mp 240-243°.

A chromatographically and spectroscopically identical sample of **1f** obtained from an exploratory reaction was dried in vacuum at 70° for 16 hours, mp 240-243°; ir ν 3450 and 3340 (NH, NH_2), 1650 broad (C=O, C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 10,300), 250 (9,080), 222 (30,600); (methanol): λ max 302 nm (ε 9,520), 250 (9,090), 222 (29,500); (*p*H 11): λ max 292 nm (ε 8,440), 248 (8,290), 226 (12,900); ^{1}H nmr: δ 0.947 (t, 3 H, CH_3), 2.14-2.22 (m, 2 H, CH_3CH_2), 4.38 (d, 2 H, NCH_2), 5.35-5.40 (m, 1 H, CHCH), 5.52-5.58 (m, 1 H, CHCH), 6.87 (br s, 2 H, NH_2), and 11.1 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S \cdot 0.1H_2O$ (254.1): C, 47.21; H, 4.84; N, 22.04. Found: C, 46.94; H, 4.70; N, 22.29.

The product **1f**, as standardly obtained from commercial (*Z*)-

1-bromo-2-pentene and as used for the syntheses of **2c** and **2d**, contained approximately 5% of the corresponding *E* olefin. For biological comparison to **1g**, a sample of **1f** was separated from its opposite geometric isomer by preparative hplc as described for **1g**. The sample of **1f** thus obtained contained less than 0.6% of **1g**.

5-Amino-3-[(*E*)-2-penten-1-yl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1g**).

The title compound was prepared, as described for **1f**, from **1e** (0.44 g, 2.39 mmoles) and (*E*)-1-bromo-2-pentene [8] (predominantly the *E* olefin, 0.32 ml, 2.68 mmoles); 0.125 g (0.495 mmole, 20%). A portion of this material was purified (less than 0.6% of **1f**) by reverse phase preparative hplc using a linear gradient of acetonitrile (0-30%) in water as the mobile phase; mp 261-263°; ir: ν 3470 and 3330 (NH, NH_2), 1710 (C=O), 1665 (C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,500), 250 (8,540), 222 (29,000); (methanol): λ max 302 nm (ε 9,110), 250 (8,730), 222 (29,300); (*p*H 11): λ max 292 nm (ε 7,540), 248 (7,540), 226 (10,200); ^1H nmr: δ 0.905 (t, 3 H, CH_3), 1.95-2.02 (m, 2 H, CH_3CH_2), 4.30 (d, 2 H, NCH_2), 5.43-5.50 (m, 1 H, CHCH), 5.57-5.64 (m, 1 H, CHCH), 6.93 (br s, 2 H, NH_2), and 11.15 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (252.3): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.50; H, 4.75; N, 21.84.

5-Amino-3-(3-methyl-2-buten-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1h**).

A mixture of **1e** (0.71 g, 3.85 mmoles), anhydrous *N,N*-dimethylformamide (40 ml), and sodium hydride (80% dispersion in mineral oil, 0.117 g, 3.9 mmoles) was stirred at ambient temperature for 1 hour. 4-Bromo-2-methyl-2-butene (0.45 ml, 3.9 mmoles) was added and the mixture was heated at 75 ±3° for 4.5 hours. The mixture was evaporated and then water (50 ml) was added. The solid produced was collected by filtration and dried in vacuum over phosphorus pentoxide for 16 hours. The solid was stirred with methanol (50 ml). Silica gel (10 g) was added and the mixture was evaporated. The dry powder was placed on a silica gel column (5.5 x 11.5 cm). The column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (1, 1), (2, 1), (3, 1), (5, 2). Eluate containing the homogeneous product was evaporated and the residual solid was crystallized from a methanol-water mixture, then dried in vacuum at 80° for 16 hours, 0.48 g (1.9 mmoles, 49%), mp 290-292°; ir: ν 3440, 3300, and 3190 (NH, NH_2), 1640 broad (C=O, C=C, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,640), 250 (8,230), 222 (28,200); (methanol): λ max 302 nm (ε 9,080), 250 (8,480), 222 (28,500); (*p*H 11): λ max 292 nm (ε 7,920), 250 (7,880), 224 (11,500); ^1H nmr: δ 1.67 (s, 3 H, CH_3), 1.75 (s, 3 H, CH_3), 4.33 (d, 2 H, CH_2), 5.19-5.22 (m, 1 H, CH), 6.91 (br s, 2 H, NH_2), and 11.12 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (252.3): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.71; H, 4.69; N, 21.86.

5-Amino-3-(4-methyl-3-penten-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1i**).

The title compound was prepared, as described for **1h**, from **1e** (0.44 g, 2.28 mmoles) and 5-bromo-2-methyl-2-pentene (0.35 ml, 2.61 mmoles) with a reaction time of 4 hours. The reaction product was purified by flash column (5.5 x 20 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 2), and then dried in vacuum over phosphorus pentoxide at 80° for 16 hours; 0.39 g (1.46 mmoles, 64%), mp 240-242° (with prior softening and sintering); ir: ν 3430 and 3220 (NH, NH_2), 1690 and 1670 (C=O, C=N), 1650 and 1630 (C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 8,520), 248 (7,720), 222 (22,100); (methanol): λ max 304 nm (ε 8,400), 250 (7,920), 222 (24,200); (*p*H 11): λ max 292 nm (ε 6,730), 248 (6,770), 226 (11,400); ^1H nmr: δ 1.52 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 2.30-2.36 (m, 2 H, CH_2), 3.75 (t, 2 H, NCH_2), 5.06-5.10 (m, 1 H, CH), 6.94 (br s, 2 H, NH_2), and 11.12 (br s, 1 H, NH).

Anal. Calcd. for $C_{11}H_{14}N_4O_2S$ (266.32): C, 49.61; H, 5.30; N, 21.04. Found: C, 49.57; H, 5.30; N, 20.72.

5-Amino-3-(4-penten-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1j**).

The title compound was prepared, as described for **1h**, from **1e** (0.39 g, 2.12 mmoles) and 5-bromo-1-pentene (0.26 ml, 2.2 mmoles) with a reaction time of 2 hours. The product was crystallized from a methanol-water mixture and dried in vacuum at 80° for 16 hours, 0.3 g (1.19 mmoles, 56%), mp 240-243°; ir: ν 3480, 3380, and 3360 (NH, NH_2), 1730 (C=O) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,500), 250 (8,130), 222 (28,400); (methanol): λ max 302 nm (ε 9,010), 248 (8,410), 222 (28,100); (*p*H 11): λ max 292 nm (ε 7,760), 250 (7,490), 226 (11,600); ^1H nmr: δ 1.68-1.75 (m, 2 H, CH_2), 2.00-2.06 (m, 2 H, CH_2), 3.75 (t, 2 H, NCH_2), 4.97 (d, 1 H, 0.5 $CHCH_2$), 5.05 (d, 1 H, 0.5 $CHCH_2$), 5.78-5.85 (m, 1 H, CH_2CH), 6.91 (br s, 2 H, NH_2), and 11.09 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (252.3): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.88; H, 4.83; N, 22.00.

5-Amino-3-(2-cyclohexen-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1k**).

The title compound was prepared, as described for **1h**, from **1e** (0.95 g, 5.16 mmoles) and 3-bromocyclohexene (1.03 ml, 8.93 mmoles) with a reaction time of 7 hours. The product was purified by flash column (5.5 x 18 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (1, 2), (2, 2), (3, 2), (5, 1), followed by crystallization from a methanol-water mixture. The product was dried in vacuum at 80° for 16 hours, 0.061 g (0.231 mmole, 4%), mp >270° dec; ir: ν 3420, 3330, and 3220 (NH, NH_2), 1715 (C=O), 1675 (C=C, C=N) cm^{-1}; uv (*p*H 1): λ max 304 nm (ε 9,500), 250 (8,260), 222 (27,800); (methanol): λ max 304 nm (ε 9,150), 248 (8,570), 222 (29,100); (*p*H 11): λ max 294 nm (ε 7,740), 250 (7,490), 224 (11,800); ^1H nmr: δ 1.58-1.60 (m, 1 H, 0.5 CH_2), 1.77-1.79 (m, 1 H, 0.5 CH_2), 1.87-1.90 (m, 1 H, 0.5 CH_2), 2.02-2.07 (m, 2 H, CH_2), 2.15-2.21 (m, 1 H, 0.5 CH_2), 5.06-5.09 (m, 1 H, NCH), 5.54 (d, 1 H, CHCH), 5.80-5.83 (m, 1 H, CHCH), 6.88 (br s, 2 H, NH_2), and 11.11 (br s, 1 H, NH).

Anal. Calcd. for $C_{11}H_{12}N_4O_2S$ (264.31): C, 49.99; H, 4.58; N, 21.20. Found: C, 49.88; H, 4.59; N, 20.86.

5-Amino-3-(cinnamyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1l**).

The title compound was prepared, as described for **1h**, from **1e** (0.5 g, 2.71 mmoles) and cinnamyl bromide (0.45 ml, 3.04

mmoles) with a reaction temperature of 80 ±3° for 4.25 hours. The product was purified by flash column (3.5 x 22 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 1), (10, 1), followed by crystallization from a methanol-water mixture. The product was dried in vacuum at 90° for 2 days, 0.191 g (0.636 mmole, 23%), mp >300°; ir: ν 3430, 3340, and 3220 (NH, NH_2), 1715 (C=O), 1680 (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 8,880), 252 (24,000), 218 (32,200); (methanol): λ max 302 nm (ε 9,040), 252 (26,400), 218 (33,600); (*p*H 11): λ max 292 nm (ε 8,600), 250 (24,400), 224 (15,400); ^1H nmr: δ 4.54 (d, 2 H, CH_2), 6.27-6.34 (m, 1 H, CH_2CH), 6.48 (d, J = 16 Hz, 1 H, CHCHAr), 6.90 (br s, 2 H, NH_2), 7.22-7.41 (m, 5 H, 5 ArH), and 11.1 (br s, 1 H, NH).

Anal. Calcd. for $C_{14}H_{12}N_4O_2S$ (300.34): C, 55.99; H, 4.03; N, 18.65. Found: C, 56.14; H, 4.00; N, 18.41.

5-Amino-3-(2-pentyn-1-yl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1m**).

The title compound was prepared, as described for **1h**, from **1e** (0.5 g, 1.71 mmoles) and 1-bromo-2-pentyne [6] (0.445 g, 3.03 mmoles) with a reaction temperature of 75 ±3° for 4 hours. The product was purified by crystallization from a methanol-water mixture and then dried in vacuum at 120° for 18 hours, 0.11 g (0.44 mmole, 25%), mp 294-298° dec; ir: ν 3410, 3310, and 3210 (NH, NH_2), 2230 (C≡C), 1700 (C=O), 1670 (C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,390), 250 (8,880), 218 (28,900); (methanol): λ max 302 nm (ε 9,140), 248 (9,010), 218 (28,100); (*p*H 11): λ max 292 nm (ε 7,370), 248 (7,780), 224 (7,820); ^1H nmr: δ 1.03 (t, 3 H, CH_3), 2.13-2.19 (m, 2 H, CH_2), 4.49 (dd, 2 H, NCH_2), 6.93 (br s, 2 H, NH_2), and 11.04 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{10}N_4O_2S$ (250.28): C, 47.99; H, 4.03; N, 22.39. Found: C, 48.05; H, 4.00; N, 21.99.

5-Amino-3-(pentan-3-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1n**).

The title compound was prepared, as described for **1h**, from **1e** (0.425 g, 2.31 mmoles) and 3-bromopentane (0.3 ml, 2.42 mmoles) with a reaction temperature of 75 ±3° for 3 hours and then 100 ±3° for 3 hours. The product was purified by flash column (5.5 x 25 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 0.5), (2, 0.5), (4, 1), and then dried in vacuum at 70° for 2 days; 0.105 g (0.429 mmole, 18%), mp >232° (broad); ir: ν 3410, 3330, and 3230 (NH, NH_2), 1700-1600 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 304 nm (ε 9,530), 250 (8,050), 224 (30,300); (methanol): λ max 304 nm (ε 9,100), 250 (8,310), 222 (27,400); (*p*H 11): λ max 294 nm (ε 7,120), 248 (7,060), 224 (11,700); ^1H nmr: δ 0.752 (t, 6 H, 2 CH_3), 1.70-1.76 (m, 2 H, CH_2), 2.08 (br s, 2 H, CH_2), 4.28 (br s, 1 H, NCH), 6.84 (br s, 2 H, NH_2), and 11.10 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{14}N_4O_2S$ (254.31): C, 47.23; H, 5.55; N, 22.03. Found: C, 47.35; H, 5.59; N, 21.75.

5-Amino-3-(nonan-5-yl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1o**).

The title compound was prepared, as described for **1h**, from **1e** (0.44 g, 2.39 mmoles) and 5-bromononane [7] (0.55 g, 2.66 mmoles) with a reaction time of 4 hours. The product was purified by flash column (3.5 x 24 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 0.5), (2, 0.5), (5, 1), and then the product was dried in vacuum at 70° for 2 days, 0.12 g (0.386 mmole, 16%), mp >175° (broad); ir: ν 3490, 3440, 3320, and 3220 (NH, NH_2), 1700-1600 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 304 nm (ε 9,840), 250 (8,190), 224 (29,900); (methanol): λ max 304 nm (ε 9,130), 250 (8,410), 224 (28,000); (*p*H 11): λ max 294 nm (ε 7,520), 250 (7,360), 224 (12,600); ^1H nmr: δ 0.809 (t, 6 H, 2 CH_3), 1.04-1.30 (m, 8 H, 4 CH_2), 1.61-1.69 (m, 2 H, CH_2), 2.04 (br s, 2 H, CH_2), 4.41 (br s, 1 H, NCH), 6.84 (br s, 2 H, NH_2), and 11.11 (s, 1 H, NH).

Anal. Calcd. for $C_{14}H_{22}N_4O_2S$ (310.42): C, 54.17; H, 7.14; N, 18.05. Found: C, 54.26; H, 7.17; N, 17.73.

5-Amino-3-(undecan-6-yl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1p**).

The title compound was prepared, as described for **1h**, from **1e** (0.46 g, 2.5 mmoles) and 6-bromoundecane [7] (0.6 g, 2.55 mmoles) with a reaction temperature of 100 ±3° for 4 hours. The product was purified by flash column (3.5 x 30 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 0.5), (1, 0.5), (2, 1), (4, 1), and then dried in vacuum at 70° for 2 days; 220 mg (0.65 mmole, 26%), mp >195° (broad). A portion (190 mg) of the solid was crystallized from a methanol-water mixture and dried at 80° under vacuum for 16 hours, 0.12 g, mp 202-204°; ir: ν 3390, 3320, and 3210 (NH, NH_2), 1655 (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 308 nm (ε 9,320), 250 (9,260), 226 (19,900); (methanol): λ max 304 nm (ε 9,470), 250 (8,490), 224 (27,000); (*p*H 11): λ max 294 nm (ε 7,640), 250 (7,470), 224 (12,500); ^1H nmr: δ 0.805 (t, 6 H, 2 CH_3), 1.12-1.23 (m, 12 H, 6 CH_2), 1.61-1.68 (m, 2 H, CH_2), 2.10 (br s, 2 H, CH_2), 4.43 (br s, 1 H, CH), 6.77 (br s, 2 H, NH_2), and 11.01 (br s, 1 H, NH).

Anal. Calcd. for $C_{16}H_{26}N_4O_2S$ (338.48): C, 56.78; H, 7.74; N, 16.55. Found: C, 56.86; H, 7.85; N, 16.45.

5-Amino-3-(tetrahydrofurfuryl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1q**).

The title compound was prepared, as described for **1h**, from **1e** (0.45 g, 2.44 mmoles) and tetrahydrofurfuryl chloride (0.28 ml, 2.57 mmoles) at a reaction temperature of 140 ±3° for 1 day. The product was purified by flash column (5.5 x 19 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 0.5), (1, 0.5), (2, 0.5), (4, 2), (6, 1), (10, 1), and then dried in vacuum at 80° for 2 days; 0.19 g (0.708 mmole, 29%). A portion (0.16 g) of the solid was crystallized from a methanol-water mixture and dried in vacuum at 80° for 16 hours; 0.11 g, mp 266-268°; ir: ν 3360 and 3110 (NH, NH_2), 1710 (C=O), 1680-1630 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,610), 250 (8,350), 222 (28,500); (methanol): λ max 302 nm (ε 9,540), 250 (8,800), 222 (28,100); (*p*H 11): λ max 292 nm (ε 7,810), 250 (7,860), 224 (10,600); ^1H nmr: δ 1.61-1.66 (m, 1 H, 0.5 CH_2), 1.79-1.92 (m, 3 H, 1.5 CH_2), 3.59-3.63 (m, 2 H, NCH_2), 3.65-3.70 (m, 1 H, 0.5 OCH_2), 3.74-3.87 (m, 1 H, 0.5 OCH_2), 4.17-4.22 (m, 1 H, OCH), 6.85 (br s, 2 H, NH_2), and 11.02 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_3S$ (268.30): C, 44.77; H, 4.51; N, 20.88. Found: C, 44.86; H, 4.57; N, 20.58.

5-Amino-3-(4-cyanobutyl)thiazolo[4,5-*d*]pyrimidine-2,7-

(3*H*,6*H*)-dione (**1r**).

The title compound was prepared, as described for **1h**, from **1e** (0.41 g, 2.33 mmoles) and 5-bromovaleronitrile (0.29 ml, 2.48 mmoles) with a reaction time of 5 hours. The product was purified by flash column (5.5 x 20 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 2.5), and then dried in vacuum at 80° for 16 hours, 0.42 g (1.58 mmoles, 68%), mp 240-243°; ir: ν 3430, 3330, and 3220 (NH, NH_2), 2250 (C≡N), 1710 (C=O), 1665 (C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,530), 250 (8,420), 222 (30,100); (methanol): λ max 302 nm (ε 9,040), 250 (8,640), 220 (28,800); (*p*H 11): λ max 292 nm (ε 7,660), 250 (7,610), 224 (10,300); ^1H nmr: δ 1.51-1.56 (m, 2 H, CH_2), 1.68-1.73 (m, 2 H, CH_2), 2.55 (t, 2 H, CH_2CN), 3.79 (t, 2 H, NCH_2), 6.93 (br s, 2 H, NH_2), and 11.1 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{10}H_{11}N_5O_2S$ (265.3): C, 45.27; H, 4.18; N, 26.40. Found: C, 45.67; H, 4.09; N, 25.97.

5-Amino-3-[4-(ethoxycarbonyl)butyl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1s**).

The title compound was prepared, as described for **1h**, from **1e** (0.4 g, 2.17 mmoles) and ethyl 5-bromovalerate (0.37 ml, 2.34 mmoles) with a reaction time of 5 hours. The product was purified by flash column (5.5 x 21 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 2.5), and then dried under vacuum at 80° for 16 hours, 0.41 g (1.31 mmoles, 60%), mp 171-174°; ir: ν 3410, 3315, 3215, and 3160 (NH, NH_2), 1730 (C=O), 1680 (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,550), 250 (8,330), 222 (30,300); (methanol): λ max 302 nm (ε 9,130), 250 (8,640), 222 (29,800); (*p*H 11): λ max 292 nm (ε 7,730), 250 (7,680), 224 (11,100); ^1H nmr: δ 1.15 (t, 3 H, CH_3), 1.47-1.50 (m, 2 H, CH_2), 1.61-1.63 (m, 2 H, CH_2), 2.32 (t, 2 H, CH_2), 3.76 (t, 2 H, NCH_2), 4.03 (q, 2 H, OCH_2), 6.92 (br s, 2 H, NH_2), and 11.1 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{12}H_{16}N_4O_4S$ (312.35): C, 46.14; H, 5.16; N, 17.94. Found: C, 46.36; H, 5.06; N, 17.59.

5-Amino-3-(benzyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**1t**).

The title compound was prepared, as described for **1h**, from **1e** (0.435 g, 2.36 mmoles) and benzyl bromide (0.3 ml, 2.52 mmoles) with a reaction time of 5 hours. The product was purified by two crystallizations from a methanol-water mixture and then dried in vacuum at 90° for 16 hours, 0.2 g (0.729 mmole, 31%), mp >320°; ir: ν 3440 and 3330 (NH, NH_2), 1640 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,570), 250 (8,650), 220 (28,500); (methanol): λ max 302 nm (ε 9,100), 250 (8,740), 222 (28,000); (*p*H 11): λ max 292 nm (ε 7,640), 250 (7,790), 224 (9,980); ^1H nmr: δ 4.96 (s, 2 H, CH_2), 6.84 (br s, 2 H, NH_2), 7.28-7.33 (m, 5 H, 5 Ar*H*), and 11.03 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{12}H_{10}N_4O_2S$ (274.3): C, 52.55; H, 3.67; N, 20.43. Found: C, 52.34; H, 3.66; N, 20.30.

5-Amino-3-(4-bromobenzyl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1u**).

The title compound was prepared, as described for **1h**, from **1e** (0.43 g, 2.33 mmoles) and 4-bromobenzyl bromide (0.6 g, 2.4 mmoles) with a reaction time of 4 hours. The product was purified by crystallization from a methanol-water mixture and dried in vacuum at 80° for 18 hours, 0.43 g (1.22 mmoles, 52%), mp >320°; ir: ν 3420, 3320, and 3210 (NH, NH_2), 1710 (C=O), 1675 (C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,100), 248 (8,200), 221 (33,200); (methanol): λ max 302 nm (ε 9,200), 248 (9,000), 221 (35,400); (*p*H 11): λ max 292 nm (ε 7,870), 248 (8,090), 228 (15,500); ^1H nmr: δ 4.92 (s, 2 H, CH_2), 6.85 (br s, 2 H, NH_2), 7.25 (d, 2 H, 2 Ar*H*), 7.51 (d, 2 H, 2 Ar*H*), and 11.03 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{12}H_9BrN_4O_2S$ (353.2): C, 40.81; H, 2.57; Br, 22.62; N, 15.86. Found: C, 40.81; H, 2.47; Br, 22.74; N, 15.64.

5-Amino-3-(4-chlorobenzyl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1v**).

The title compound was prepared, as described for **1h**, from **1e** (0.43 g, 2.33 mmoles) and 4-chlorobenzyl chloride (0.32 ml, 2.5 mmoles) with a reaction time of 8 hours. The product was purified by two crystallizations from a methanol-water mixture and dried in vacuum at 90° for 16 hours, 0.053 g (0.172 mmole, 7%), mp >320°; ir: ν 3420, 3320, and 3210 (NH, NH_2), 1710 (C=O), 1675 (C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 6,630), 248 (4,460), 222 (17,700); (methanol): λ max 302 nm (ε 7,100), 248 (6,820), 222 (27,000); (*p*H 11): λ max 292 nm (ε 5,950), 248 (6,040), 226 (12,400); ^1H nmr: δ 4.95 (s, 2 H, CH_2), 6.88 (br s, 2 H, NH_2), 7.32 (d, 2 H, 2 Ar*H*), 7.39 (d, 2 H, 2 Ar*H*), and 11.07 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{12}H_9ClN_4O_2S$ (308.75): C, 46.68; H, 2.94; Cl, 11.48; N, 18.15. Found: C, 46.58; H, 2.85; Cl, 11.38; N, 17.97.

5-Amino-3-(4-cyanobenzyl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**1w**).

The title compound was prepared, as described for **1h**, from **1e** (0.44 g, 2.39 mmoles) and α-bromo-*p*-tolunitrile (0.50 g, 2.55 mmoles) with a reaction time of 4 hours. The product was purified by crystallization from a methanol-water mixture. The solid was dried in vacuum at 80° for 16 hours, 0.42 g (1.38 mmoles, 58%), mp >320°; ir: ν 3490, 3390, and 3210 (NH, NH_2), 2230 (C≡N), 1670 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 10,000), 252 sh (9,900), 222 (36,800); (methanol): λ max 302 nm (ε 9,530), 252 sh (9,890), 222 (35,000); (*p*H 11): λ max 292 nm (ε 8,110), 232 (26,000); ^1H nmr: δ 5.04 (s, 2 H, CH_2), 6.85 (br s, 2 H, NH_2), 7.46 (d, 2 H, 2 Ar*H*), 7.78 (d, 2 H, 2 Ar*H*), and 11.05 (br s, 1 H, N*H*).

Anal. Calcd. for $C_{13}H_9N_5O_2S \cdot 0.25H_2O$ (303.82): C, 51.39; H, 3.15; N, 23.05. Found: C, 51.55; H, 3.08; N, 22.89.

5-Amino-3-(4-carboxybutyl)thiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (**2a**).

A mixture of **1s** (0.312 g, 1 mmole) and 1 *M* sodium hydroxide (5 ml) was stirred at ambient temperature for 1.5 hours. The mixture was acidified (*p*H 4) with acetic acid and then refrigerated (5°) for 16 hours. The solid was collected by filtration, crystallized from a methanol-water mixture, and dried under vacuum at 80° for 16 hours; 0.22 g (0.774 mmole, 77%), mp 263-265°; ir: ν 3410, 3315, and 3180 (NH, NH_2), 1725 (C=O), 1640 (C=O, C= N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,790), 250 (8,520), 222 (31,000); (methanol): λ max 302 nm (ε 9,480), 250 (8,790), 222 (30,400); (*p*H 11): λ max 292 nm (ε 8,260), 250 (8,150), 224 (12,200); ^1H nmr: δ 1.43-1.50 (m, 2 H, CH_2),

1.60-1.67 (m, 2 H, CH_2), 2.24 (t, 2 H, CH_2), 3.76 (t, 2 H, NCH_2), 6.91 (br s, 2 H, NH_2), 11.1 (br s, 1 H, NH), and 12.0 (br s, 1 H, CO_2H).

Anal. Calcd. for $C_{10}H_{12}N_4O_4S$ (284.3): C, 42.25; H, 4.25; N, 19.71. Found: C, 42.32; H, 4.22; N, 19.69.

5-Amino-3-[4-(carbamoyl)butyl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**2b**).

A mixture of **1s** (0.34 g, 1.09 mmoles), concentrated ammonium hydroxide (25 ml), and methanol (10 ml) was stirred at ambient temperature for 2 days. The mixture was acidified (*p*H 6) with acetic acid and then evaporated in vacuum. The residue was coevaporated with ethanol (2 x 50 ml) and then stirred with methanol (50 ml). Silica gel (10 g) was added and the mixture was evaporated. The dry powder was placed on a silica gel column (5.5 x 19 cm) and the column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (1, 0.5), (2, 0.5), (5, 2), (7, 1), (10, 1), (25, 1). The eluate containing the homogeneous product was evaporated. The solid residue was washed with water (20 ml), crystallized from a methanol-water mixture, and dried in vacuum at 90° for 16 hours, 0.089 g (0.314 mmole, 29%), mp 250-252°; ir: ν 3450, 3380, 3350, and 3200 (NH, NH_2), 1680 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 302 nm (ε 9,790), 250 (8,510), 222 (30,300); (methanol): λ max 302 nm (ε 9,330), 250 (8,720), 222 (29,700); (*p*H 11): λ max 292 nm (ε 7,760), 250 (7,630), 224 (11,000); ^1H nmr: δ 1.44-1.52 (m, 2 H, CH_2), 1.58-1.67 (m, 2 H, CH_2), 2.05-2.1 (m, 2 H, CH_2CO), 3.73-3.78 (m, 2 H, NCH_2), 6.54 [br s, 1 H, 0.5 $C(O)NH_2$], 6.80 (br s, 2 H, NH_2), 7.09 [br s, 1 H, 0.5 $C(O)NH_2$], and 10.97 (br s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{13}N_5O_3S$ (283.31): C, 42.39; H, 4.63; N, 24.72. Found: C, 42.37; H, 4.64; N, 24.44.

5-Amino-6-methyl-3-[(*Z*)-2-penten-1-yl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**2c**).

A mixture of **1f** (0.35 g, 1.35 mmoles), sodium hydride (80% dispersion in mineral oil, 0.42 g, 1.4 mmoles), and anhydrous *N,N*-dimethylformamide (15 ml) was protected from moisture and stirred at ambient temperature for 0.25 hour. Iodomethane (0.1 ml, 1.61 mmoles) was added and the mixture was stirred for an additional 1 hour. The mixture was evaporated in vacuum and then water (20 ml) was added. The mixture was stirred for 15 minutes and the solid was collected by filtration. The solid was suspended in methanol (25 ml) and silica gel (5 g) was added. The mixture was evaporated and the dry powder was placed on a silica gel column (5.5 x 20 cm). The column was flash eluted with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1). The eluate containing the homogeneous product was evaporated and the solid was dried in vacuum at 80° for 16 hours, 0.24 g (0.901 mmole, 66%), mp 184-187°; ir: ν 3420, 3330, and 3210 (NH_2), 1715 (C=O), 1675 (C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 300 nm (ε 9,370), 252 (8,020), 222 (30,900); (methanol): λ max 302 nm (ε 9,070), 252 (8,350), 222 (31,300); (*p*H 11): λ max 300 nm (ε 9,410), 252 (8,080), 226 (21,000); ^1H nmr: δ 0.954 (t, 3 H, CH_3), 2.15-2.22 (m, 2 H, CH_2), 3.29 (s, 3 H, CH_3), 4.38 (d, 2 H, NCH_2), 5.34-5.40 (m, 1 H, CHCH), 5.53-5.59 (m, 1 H, CHCH), and 7.51 (br s, 2 H, NH_2).

Anal. Calcd. for $C_{11}H_{14}N_4O_2S$ (266.32): C, 49.61; H, 5.30; N, 21.04. Found: C, 49.93; H, 5.17; N, 20.77.

5,6-Diamino-3-[(*Z*)-2-penten-1-yl]thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**2d**).

A mixture of **1f** (0.50 g, 2.0 mmoles) and 1 *M* sodium hydroxide (6 ml) was stirred to dissolve the solid. A solution of hydroxylamine-*O*-sulfonic acid (0.34 g, 3.1 mmoles) in water (4 ml) was added and the resulting mixture was stirred at ambient temperature for 1 day. The solid was collected by filtration and then stirred with a mixture of concentrated ammonium hydroxide (5 ml) and water (20 ml) for 1 hour. The solid was again collected, crystallized from a methanol-water mixture, and dried in vacuum at 80° for 18 hours, 0.15 g (0.561 mmole, 28%), mp 137-139°; ir: ν 3480, 3370, and 3310 (NH_2), 1715 (C=O), 1685 (C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 300 nm (ε 9,440), 248 (7,780), 222 (30,300); (methanol): λ max 300 nm (ε 8,900), 248 (8,110), 222 (29,900); (*p*H 11): λ max 300 nm (ε 9,680), 250 (8,190), 226 (20,600); ^1H nmr: δ 0.958 (t, 3 H, CH_3), 2.18-2.23 (m, 2 H, CH_3CH_2), 4.39 (d, 2 H, NCH_2), 5.3-5.5 (m, 3 H, CHCH and NNH_2), 5.55-5.65 (m, 1 H, CHCH), and 7.42 (br s, 2 H, NH_2).

Anal. Calcd. for $C_{10}H_{13}N_5O_2S$ (267.31): C, 44.93; H, 4.90; N, 26.20. Found: C, 45.06; H, 4.89; N, 26.19.

5-Amino-2-hexylaminothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**4**).

A mixture of 5-amino-2-chlorothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one [5] (**3**, 0.41 g, 2.02 mmoles), hexylamine (1.3 ml), and water (40 ml) was stirred and heated at reflux for 4.5 hours. After cooling to ambient temperature, the solid was collected and washed with water (20 ml) followed by acetone (20 ml). The solid was crystallized from a *N,N*-dimethylformamide-water mixture and then suspended in a mixture of water (100 ml) and ethanol (100 ml). The mixture was stirred and heated at boiling for 0.25 hour. The hot mixture was filtered, the solid was washed with water (25 ml) followed by acetone (25 ml), and dried at 80° in vacuum for 16 hours, 0.41 g (1.44 mmoles, 71%), mp >320°; ir: ν 3650, 3530, 3460, 3280, and 3130 (NH, NH_2), 1650 broad (C=O, C=N) cm^{-1}; uv (*p*H 1): λ max 312 nm (ε 16,500), 260 sh (6,810), 228 (26,600); (*p*H 11): λ max 304 nm (ε 11,300), 236 (33,100); ^1H nmr: δ 0.87 (t, 3 H, CH_3), 1.29-1.36 (m, 8 H, 4 CH_2), 1.52-1.59 (m, 2 H, CH_2N), 6.37 (br s, 2 H, NH_2), 8.42 (br s, 1 H, CH_2NH), and 10.69 (br s, 1 H, NH).

Anal. Calcd. for $C_{11}H_{17}N_5OS \cdot H_2O$ (285.37): C, 46.30; H, 6.71; N, 24.54. Found: C, 46.36; H, 6.72; N, 24.57.

2-Amino-4-[(*Z*)-2-pentenyl]thiazolo[4,5-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (**6a**).

The title compound was prepared, as described for **1h**, from **5** [5] (0.49 g, 2.66 mmoles) and 1-bromo-2-pentene (predominately the *Z* olefin, 0.34 ml, 2.87 mmoles) at a reaction temperature of 80 ±3° for 4.5 hours. The product was purified by flash column (5.5 x 17.5 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 1), (10, 1), followed by crystallization from a methanol-water mixture. The solid was dried in vacuum at 100° for 16 hours, 0.127 g (0.503 mmole, 19%), mp 227-229°; ir: ν 3450, 3370, and 3160 (NH, NH_2), and 1660 broad (C=O, C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 310 nm (ε 15,300), 226 (24,300); (methanol): λ max 310 nm (ε 14,900), 226 (23,500); (*p*H 11): λ max 306 nm (ε 13,700), 242 sh (11,100), 226 (18,700); ^1H nmr: δ 0.961 (t, 3 H, CH_3), 2.15-2.23 (m, 2 H, CH_2), 4.55 (d, 2 H, CH_2), 5.36-5.41 (m, 1 H, CH), 5.49-5.54 (m, 1 H, CH), 8.44 (s, 2 H, NH_2), and 11.04 (s, 1 H, NH).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (252.3): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.60; H, 4.72; N, 22.11.

2-Amino-4-(3-methyl-2-butenyl)thiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (**6b**).

The title compound was prepared, as described for **1h**, from **5** [5] (0.47 g, 2.55 mmoles) and 4-bromo-2-methyl-2-butene (0.32 ml, 2.78 mmoles) at a reaction temperature of 80 ±3° for 4 hours. The product was purified by flash column (5.5 x 21 cm) chromatography, eluting with progressively increasing concentrations of methanol in dichloromethane (% methanol, volume in l): (0, 1), (1, 1), (2, 1), (3, 1), (5, 2), followed by crystallization from a methanol-water mixture. The solid was dried in vacuum at 90° for 2 days, 0.12 g (0.476 mmole, 19%), mp 273-275°; ir: ν 3480, 3300, and 3150 (NH, NH_2), 1710 (C=O), and 1650 broad (C=O, C=N, C=C) cm^{-1}; uv (*p*H 1): λ max 310 nm (ε 15,100), 226 (23,900); (methanol): λ max 310 nm (ε 14,600), 226 (22,700); (*p*H 11): λ max 306 nm (ε 13,300), 242 sh (11,300), 226 (19,800); ^1H nmr: δ 1.67 (s, 3 H, CH_3), 1.75 (s, 3 H, CH_3), 4.49 (d, 2 H, CH_2), 5.19-5.22 (m, 1 H, C*H*), 8.45 (s, 2 H, NH_2), and 11.02 (s, 1 H, N*H*).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$ (252.3): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.66; H, 4.78; N, 22.04.

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