Structure−Activity Relationships of 7-Deaza-6-benzylthioinosine Analogues as Ligands of *Toxoplasma gondii* Adenosine Kinase

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Several 7-deaza-6-benzylthioinosine analogues with varied substituents on aromatic ring were synthesized and evaluated against *Toxoplasma gondii* adenosine kinase (EC.2.7.1.20). Structure−activity relationships indicated that the nitrogen atom at the 7-position does not appear to be a critical structural requirement. Molecular modeling reveals that the 7-deazapurine motif provided flexibility to the 6-benzylthio group as a result of the absence of H-bonding between N7 and Thr140. This flexibility allowed better fitting of the 6-benzylthio group into the hydrophobic pocket of the enzyme at the 6-position. In general, single substitutions at the para or meta position enhanced binding. On the other hand, single substitutions at the ortho position led to the loss of binding affinity. The most potent compounds, 7-deaza-*p*-cyano-6-benzylthioinosine (IC_{50} = 5.3 μM) and 7-deaza-*p*-methoxy-6-benzylthioinosine (IC_{50} = 4.6 μM), were evaluated in cell culture to delineate their selective toxicity.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. *T. gondii* infects a wide range of hosts, including human and warm-blooded animals.[1–3] Toxoplasmic infection in human is estimated to affect more than a billion individuals worldwide, resulting in an important public health problem.[4,5] *T. gondii* infection causes morbidity and mortality in immunocompromised individuals such as organ transplant, leukemia, and AIDS patients, as well as congenitally infected children. In immunocompromised patients, reactivation of chronic *T. gondii* infection can cause a severe central nervous system disease, and thus, toxoplasmic encephalitis and focal neurological lesions are the most common clinical manifestations.[6,7] Infection acquired during pregnancy can lead to congenital toxoplasmosis of the fetus, which results in abortion, neonatal death, or fetal abnormalities that may be present at birth or may develop later in life.[8,9]

At the present time, there is no vaccine available to prevent human infection with this class of pathogens.[10,11] Antifolate drugs, such as the combination of pyrimethamine and sulfadiazine, are currently used for the treatment of toxoplasmic infection.[12–15] This therapy, however, is ineffective against toxoplasma tissue cysts[6,16] and causes side effects such as bone marrow depression and skin rashes.[15,17] In order to reduce undesirable side effects, folic acid and spiramycin are used as alternative regimen of sulfa drugs.[11,18] However, the emergence of drug resistance and complications associated with long-term treatment of toxoplasmosis warrant the need for the development of new and effective chemotherapeutic agents.

Recently, *T. gondii* adenosine kinase (EC.2.7.1.20) has been investigated as an attractive chemotherapeutic target for the development of antitoxoplasmic agents.[19,20] Several differences between *T. gondii* and their host are found in purine metabolism. Unlike their host, *T. gondii* lack de novo purine synthesis and thus depend on the salvage pathways for their purine requirements. Specifically, the activity of adenosine kinase in *T. gondii* is 10-fold higher than those of other enzymes in the purine salvage pathways. Therefore, *T. gondii* adenosine kinase contributes significantly to the survival of the parasite.[20] However, deficiency of adenosine kinase was shown not to be lethal to the parasites, indicating that inhibition of the enzyme will not lead to toxicity in toxoplasma. Structure−activity relationships, comparative enzymatic, metabolic, and X-ray structural studies of *T. gondii* adenosine kinase have identified subversive substrates that are selectively metabolized to cytotoxic nucleotides by the *T. gondii* but not human adenosine kinase.[19–30]

We have previously reported that 6-benzylthioinosine analogues[19,20,22,24–26] are selective subversive substrates for the *T. gondii*, but not host, adenosine kinase. Certain 6-benzylthioinosine analogues showed potent antitoxoplasma activity in cell culture as well as in mouse infection model,[19,22,25] demonstrating the potential usefulness of these classes of compounds in the treatment of toxoplasmosis. Furthermore, the 6-benzylthioinosine analogues act as inhibitors of the mammalian nucleoside transporter ENT1 (es) in host cells,[26] thus elucidating the basis for the lack of uptake of these compounds by uninfected host cells.[23] Several N^6-benzyladenosine analogues also showed appreciable antiparasitic activity, however; they exhibited host toxicity.[27] In light of these findings,[19,22,24–27] we have chosen the 6-benzylthio group as a more suitable scaffold for further development of purine nucleoside analogues as antitoxoplasmic agents.

In our continuing effort to develop potent and selective antitoxoplasmic agents, our interest has focused on base-modified 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) nucleosides in view of their significant biological properties, including antiparasitic as well as antiviral, antimicrobial, and anticancer activities.[31–34] The base-modified nucleosides are synthetically challenging and can generate new classes of chemotherapeutic agents. 7-Deazapurine is an ideal mimic of purine in which the nitrogen atom at the 7-position is replaced by a carbon atom.

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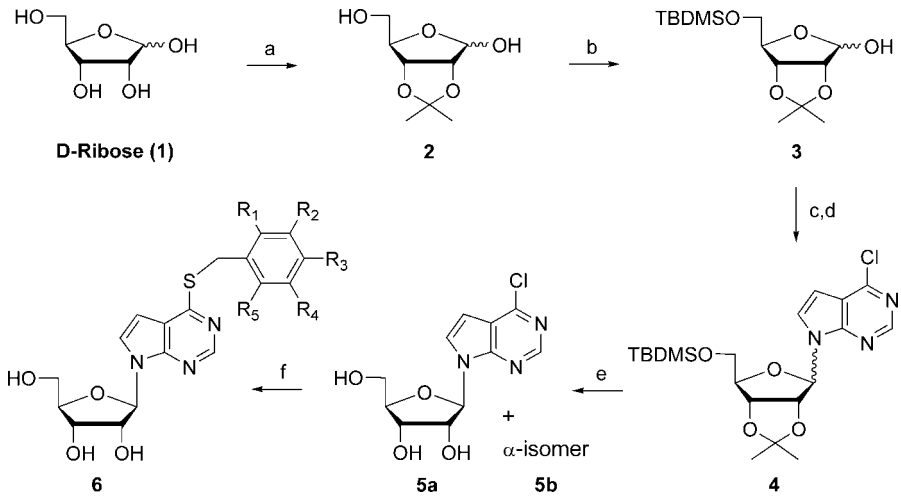
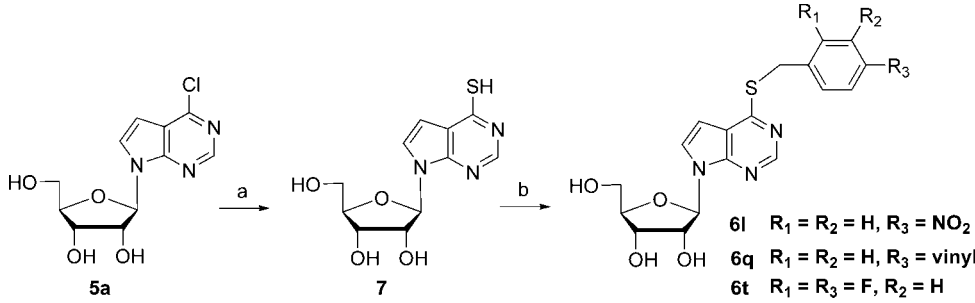
Scheme 1[a]



[a] Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH, acetone, 0 °C to room temp, 1 h, 81%; (b) TBDMSCl, imidazole, CH_2Cl_2, room temp, 2 h, 83%; (c) CCl_4, HMPT, THF, −30 °C, 1 h; (d) 6-chloro-7-deazapurine, KOH, TDA-1, THF, 16 h, 40% (two steps from **3**); (e) Dowex 50WX8-200, MeOH, room temp, 8 h, 95%; (f) appropriate benzylthiol, NaH, THF, room temp, 1 h.

Scheme 2[a]



[a] Reagents and conditions: (a) thiourea, EtOH, reflux, 3 h, 85%; (b) appropriate benzylhalide, NH_4OH/H_2O, room temp, 11 h.

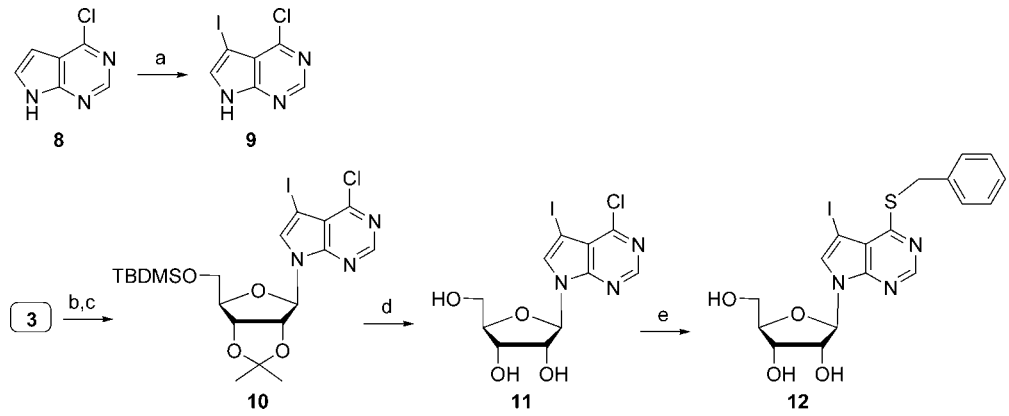
Hence, the altered character of the heterocycle might contribute to differential interactions at the binding site of *T. gondii* adenosine kinase. Therefore, the main goal of the present studies is to investigate whether or not the elimination of the N7 of inosine will enhance the binding of 6-benzylthioinosines to *T. gondii* adenosine kinase. A better understanding of substrate analogues binding would aid in the rational design of drugs that maintain activity while decreasing toxicity. Herein, we report the synthesis of novel 7-deaza-6-benzylthioinosine analogues and the structure−activity relationships of these analogues as ligands of *T. gondii* adenosine kinase.

Results and Discussion

Chemistry. Synthesis of 7-deaza-6-benzylthioinosine analogues **6a**−**w** is described in Schemes 1 and 2. D-Ribose was converted to the isopropylidene derivative **2** with 2,2-dimethoxypropane in the presence of catalytic amounts of *p*-toluenesulfonic acid in 81% yield, followed by the protection with *tert*-butyldimethylsilyl chloride that gave compound **3** in 83% yield. Stereospecific glycosylation of the protected lactol **3** with 6-chloro-7-deazapurine was conducted via an α-chloro sugar intermediate.[35–37] Compound **3** was reacted with carbon tetrachloride and hexamethylphosphorus triamide (HMPT) in tetrahydrofuran at −30 °C to generate in situ 1-chloro-2,3-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-α-D-ribofuranose. The resulting ribofuranosyl chloride was coupled with the potassium salt of 6-chloro-7-deazapurine in the presence of tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) at room temperature to give compound **4** as an α/*β* mixture in 40% yield in two steps. The phase-transfer catalyst TDA-1 increased the nucleophilicity of the 6-chloro-7-deazapurine anion by forming a complex with a potassium cation as reported in the literature.[35] The *tert*-butyldimethylsilyl and isopropylidene groups of compound **4** were removed with Dowex 50WX8-200 in methanol to give the *β*-anomer **5a** and α-anomer **5b** in good yield. In this step, the anomeric mixture was readily separated by silica gel column chromatography (*β*/α = 10:1, 95% yield). Finally, variation of the key intermediate **5a** was accomplished by treatment with the appropriate benzylthiols in the presence of sodium hydride at room temperature to give the corresponding 7-deaza-6-benzylthioinosine analogue **6** in good yields. Some of the substituted benzylthiols, which are not commercially available, have been prepared according to the reported procedures.[38] Unexpectedly, the yield of 4-nitrobenzyl analogue **6l** was low (∼10%) because of decomposition under this condition. We found that the use of thione **7** as an intermediate resulted in improved yield (Scheme 2). Treatment of triol **5a** with thiourea in ethanol gave compound **7**, which was alkylated with 4-nitrobenzyl bromide[39] to provide compound **6l** in 76% yield. In a similar reaction, we obtained a good result in the transformation of the intermediate **7** to compounds **6q** and **6t**.

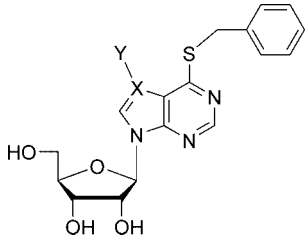
Synthesis of compound **12** was accomplished as shown in Scheme 3. The commercially available 6-chloro-7-deazapurine **8** could be directly iodinated with *N*-iodosuccinimide in DMF at the C7-position to give the 7-iodinated compound **9** utilizing a known procedure.[40] Compound **9** was coupled with 1-chloro-2,3-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-α-D-ribofuranose freshly prepared from compound **3** to give iodinated

Scheme 3[a]



[a] Reagents and conditions: (a) NIS, DMF, room temp, 2 h, 93%; (b) CCl_4, HMPT, THF, −30 °C, 1 h; (c) **9**, KOH, TDA-1, THF, 16 h, 44% (two steps from **3**); (d) Dowex 50WX8-200, MeOH, room temp, 8 h, 93%; (e) benzylthiol, NaH, THF, room temp, 1 h, 84%.

Table 1. Binding Affinities (Apparent K_i) of 7-Deaza-6-benzylthioinosine Analogues (**6a** and **12**) and 6-Benzylthioinosine to *Toxoplasma gondii* Adenosine Kinase



compd	X	Y	K_i [a] (μM)
6a	C	H	28 ± 8.6
12	C	I	82.6 ± 4.2
6-benzylthioinosine	N		37.7 ± 3.6

[a] All values represent the mean ± SD of at least two independent experiments with three replications each as previously described.[24,25,27] Since these compounds are alternative substrates of *T. gondii* adenosine kinase, their apparent K_i values are equal to their apparent K_m values.[48]

ribonucleoside **10**. In this case, the formation of α-anomer was negligible. Subsequently, compound **10** was treated with Dowex 50WX8-200 in methanol at room temperature to afford compound **11** in good yield followed by the nucleophilic displacement reaction gave 7-iodo-7-deaza-6-benzylthioinosine **12** in 84% yield.

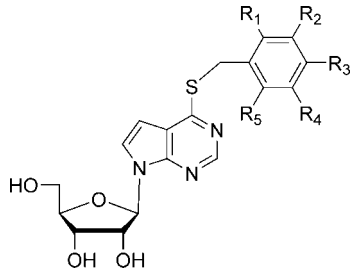
Effects of Structural Modification at the 7-Position of the Purine Ring on the Binding Affinity of 6-Benzylthioinosine. Previous structure−activity relationship[21,24,27] and crystal structure[28] studies demonstrated that two hydrophobic pockets exist on the *T. gondii* adenosine kinase around the C6 and N7 positions of the purine ring. The hydrophobic pocket at N7-position is smaller than that at C6-position.[24] Therefore, we wanted to examine the effects of eliminating the N7 of inosine, and the role of substitutions at the C6 and C7 positions on the binding affinity of the synthesized compounds.

Table 1 shows that the binding affinity (apparent K_i values) of 7-deaza-6-benzylthioinosine (**6a**, 27.9 μM) was relatively better than 6-benzylthioinosine (37.7 μM). This finding indicated that the nitrogen atom at the 7-position does not appear to be a critical structural requirement for binding to *T. gondii* adenosine kinase. Furthermore, since 7-iodo-7-deazaadenosine (7-iodotubercidin) is a better ligand than adenosine for *T. gondii* adenosine kinase,[21] we were prompted to explore the effects of adding the iodo substitution at the C7-position of 7-deaza-6-benzylthioinosine (**6a**). Unexpectedly, however, 7-iodo-7-deaza-6-benzylthioinosine (**12**) exhibited a lesser binding affinity to the enzyme (82.6 μM) than 7-deaza-6-benzylthioinosine (**6a**, 27.9 μM). The decrease in binding affinity of 7-iodo-7-deaza-6-benzylthioinosine (**12**) appears to be due to a poor interaction and ligand-fit profile in the enzyme active site, as will be discussed below.

Structure−Activity Relationships of Various Substituents on the Aromatic Ring. Structure−activity relationships were performed by varying substituents on the aromatic ring of **6a**. The binding affinities of the 7-deaza-6-benzylthioinosine analogues **6a**−**w** to *T. gondii* adenosine kinase were compared with that of the natural substrate adenosine (Table 2). The binding affinities of compounds **6h** (*m*-methyl, 8.5 μM), **6j** (*p*-chloro, 11 μM), **6m** (*p*-cyano, 5.2 μM), **6n** (*p*-methoxycarbonyl, 7.0 μM), **6s** (*p*-methoxy, 5.8 μM), and **6w** (*o*-chloro-*o*-fluoro, 7.2 μM) to *T. gondii* adenosine kinase were better than the unsubstituted compound **6a** (28 μM). Compounds **6m** (*p*-cyano, 5.2 μM) and **6s** (*p*-methoxy, 5.8 μM) were relatively bettrer than adenosine (8.9 μM).

In general, single substitutions at the ortho position (**6b**−**e**) led to the loss in binding affinity when compared to the unsubstituted compound **6a**. The weakest ligands in this series were **6e** (*o*-methyl, 227.9 μM) and **6k** (*p*-bromo, 252.7 μM). Notably, when a methyl group at the ortho position (**6e**, 227.9 μM) was moved to the meta (**6h**, 8.5 μM) or the para (**6o**, 18.5 μM) position, a substantial increase in the binding affinity was observed. At the meta position, an electron-donating substituent (**6h**, 8.5 μM) resulted in a better binding affinity than an electron-withdrawing substituent (**6f**, 44.6 μM and **6g**, 22.4 μM). At the para position, a chlorine substitution (**6j**, 11.3 μM) exhibited somewhat increased binding affinity in comparison to a fluorine substitution (**6i**, 36.7 μM). Interestingly, the bromine substitution (**6k**, 252.7 μM) displayed a dramatic decrease in binding affinity. A similar trend was also observed for halogen substitutions in the ortho-substituted compounds **6b**−**d**, and the order of the potency was Cl > F ≫ Br. In addition to electronic effects, it appears that the relative size of the halogen substituents might also play a role in how they were accommodated in the active site of *T. gondii* adenosine kinase. Compounds with a para substituent of similar size but different electoronegativity [e.g., **6r** (*p*-trifluoromethoxy 35.0 μM) vs **6s** (*p*-methoxy, 5.8 μM)] exhibited significantely different binding affinities. Compounds with different size but similar electronegativity [e.g., **6r** (*p*-trifluoromethoxy, 35.0 μM) and **6m** (*p*-cyano, 5.2 μM)] also showed significant difference in binding affinity. In fact, the modeling results suggest the different binding mode of **6r** (*p*-trifluoromethoxy) in comparison to **6s**

Table 2. Binding Affinities (Apparent K_i) of 7-Deaza-6-benzylthioinosine Analogues **6a**−**w** and Adenosine to *Toxoplasma gondii* Adenosine Kinase



compd	R_1	R_2	R_3	R_4	R_5	K_i [a] (μM)
6a	H	H	H	H	H	28 ± 8.6
6b	F	H	H	H	H	59 ± 6.1
6c	Cl	H	H	H	H	36 ± 5.7
6d	Br	H	H	H	H	106 ± 17
6e	CH_3	H	H	H	H	228 ± 49
6f	H	NO_2	H	H	H	45 ± 8.9
6g	H	CF_3	H	H	H	22 ± 4.0
6h	H	CH_3	H	H	H	8.5 ± 1.4
6i	H	H	F	H	H	37 ± 14
6j	H	H	Cl	H	H	11 ± 1.1
6k	H	H	Br	H	H	253 ± 56
6l	H	H	NO_2	H	H	35 ± 9.5
6m	H	H	CN	H	H	5.2 ± 1.1
6n	H	H	CO_2CH_3	H	H	7.0 ± 1.3
6o	H	H	CH_3	H	H	19 ± 3.9
6p	H	H	^tBu	H	H	24 ± 2.1
6q	H	H	CH=CH_2	H	H	180 ± 30
6r	H	H	OCF_3	H	H	35 ± 14
6s	H	H	OCH_3	H	H	5.8 ± 1.0
6t	F	H	F	H	H	16 ± 3.9
6u	Cl	H	Cl	H	H	26 ± 7.6
6v	H	Cl	Cl	H	H	29 ± 11
6w	F	H	H	H	Cl	7.2 ± 1.3
adenosine						8.9 ± 3.2

[a] All values represent the mean ± SD of at least two independent experiments with three replications each as previously described.[24,25,27] Since these compounds are alternative substrates of *T. gondii* adenosine kinase, their apparent K_i values are equal to their apparent K_m values.[48]

(*p*-methoxy), in which the *p*-trifluormethoxy group of **6r** orients away from the hydrophobic pocket in comparison to **6s** (*p*-methoxy). These results suggest that the binding affinity to *T. gondii* adenosine kinase is affected by both the electronic and the size of the para substituents. Previously, similar observation was made from the study of 6-benzylthioinosine analogues.[24] The best result was achieved when a cyano group (**6m**, 5.2 μM) or a methoxy group (**6s**, 5.8 μM) was added to the para position. In the case of disubstituted analogues, **6t**−**w**, di-ortho substitutions (**6w**, 7.2 μM) showed increased binding affinity in comparison to the ortho−para disubstitutions (**6t**, 16.0 μM and **6u**, 25.8 μM) or the meta−para disubstitutions (**6v**, 28.7 μM). Furthermore, the binding affinities of dihalogen derivatives (**6t**−**w**) were relatively better than the mono derivatives (**6b**, 59 μM and **6c**, 36 μM). Compounds with fluoro substituents (**6t**, 16.0 μM and **6w**, 7.2 μM) were the best ligands among the disubstituted derivatives.

These results indicate that the incorporation of the 7-deazapurine motif led to improvement in the binding affinity of the 6-benzylthioinosines. Furthermore, the functionality of the 6-benzylthio group on the 7-deazapurine moiety was extended by the addition of substituents such as *p*-cyano (**6m**) and *p*-methoxy (**6s**). Such substituents provided additional interactions with the surrounding residues in the binding pocket of *T. gondii* adenosine kinase and were conducive to enhancing the binding affinity of the 7-deaza-6-benzylthioinosines. The present results also confirm that this structural optimization process is one of the best ways to influence the binding affinity for *T. gondii* adenosine kinase as indicated by previous studies.[24,27]

Table 3. Effect of 7-Deaza-6-benzylthioinosine Analogues (**6m** and **6s**) and Therapeutic Compounds on Host Toxicity[a] and Percent Survival[b] of Wild Type (RH) and Adenosine Kinase Deficient ($TgAK^{-3}$) Strains of *Toxoplasma gondii* Grown in Human Fibroblasts in Culture

		concentration (μM)					
compd	infection	0	5	10	25	50	IC_{50} (μM)
6m (*p*-CN)	wild type (RH)	100	52.5	4.0	0.0	0.0	5.3 ± 0.6
	$TgAK^{-3}$	100	98.4	100	100	100	
	none[a]	100	100	100	94.3	82.1	
6s (*p*-OCH_3)	wild type (RH)	100	49.8	5.0	0.0	0.0	4.6 ± 0.2
	$TgAK^{-3}$	100	100	100	100	100	
	none[a]	100	100	100	100	90.6	
pyrimethamine[c]	wild type (RH)	100	55.6	36.5	13.8	5.8	5.9 ± 0.8
	none[a]	100	101	100	108	108	
sulfadiazine[c]	wild type (RH)	100	74.6	64.8	59.8	46.4	41.2 ± 1.9
	none[a]	100	98.2	99.8	99.8	102	

[a] Host toxicity of uninfected cells was measured by MTT method in at least two independent experiments each with three replications as previously described.[22–25,27] [b] Percent survival of parasites was measured by incorporation of [5,6-^{13}H]uracil in at least two independent experiments with three replications each as previously described.[22–25,27] [c] Therapeutic compounds.

Evaluation of Antitoxoplasma Activity. The two best ligands, 7-deaza-*p*-cyano-6-benzylthioinosine (**6m**) and 7-deaza-*p*-methoxy-6-benzylthioinosine (**6s**), were tested for their antitoxoplasma efficacy and host toxicity. Their IC_{50} values were compared to those obtained from the clinically used drugs pyrimethamine and sulfadiazine (Table 3). The two analogues were effective against infection with the wild type (RH) *T. gondii* in a dose-dependent manner but not against infection with the adenosine kinase deficient ($TgAK^{-3}$) strain. In agreement with their binding affinities (Table 2), **6m** and **6s** showed potent cellular activity with IC_{50} values of 5.3 and 4.6 μM, respectively. Furthermore, **6m** and **6s** exhibited close to or better potency than pyrimethamine (IC_{50} = 5.9 μM) and sulfadiazine (IC_{50} = 41.2 μM). The lack of activity against infection with the adenosine kinase deficient ($TgAK^{-3}$) strain of *T. gondii* demonstrates that these compounds are active substrates for *T. gondii* adenosine kinase in vivo as was the case in vitro (Table 2). It is also noted that **6m** and **6s** had no detectable host cell toxicity at concentrations up to 50 μM, in spite of the absence of the nitrogen atom in the 7-position (Table 3). This is noteworthy, since 7-iodo-7-deazaadenosine (7-iodotubercidin) is an extremely toxic[22] and nonselective ligand of *T. gondii* adenosine kinase.[19,20,22]

These results demonstrated that 7-deaza-6-benzylthioinosine analogues are selectively activated by *T. gondii*, but not human, adenosine kinase, which leads to the selective death of the parasite. These findings also implied that the absence of the nitrogen atom in the 7-position does not play a significant role in the antitoxoplasma effects of the 6-benzylthioinosines. These studies provided useful information to define the structural feature required for the potency and selectivity of antitoxoplasmic agents and to guide further design of such agents.

Molecular Modeling. *T. gondii* adenosine kinase consists of 363 residues (39.3 kDa).[41] It catalyzes the phosphorylation of adenosine to adenosine 5′-monophosphate (AMP), using the γ-phosphate of ATP. The overall enzyme structure[28] including recent studies[30] suggested an induced fit enzyme movement from open conformation to closed conformation for effective phosphate transfer. Our recent induced fit molecular modeling studies

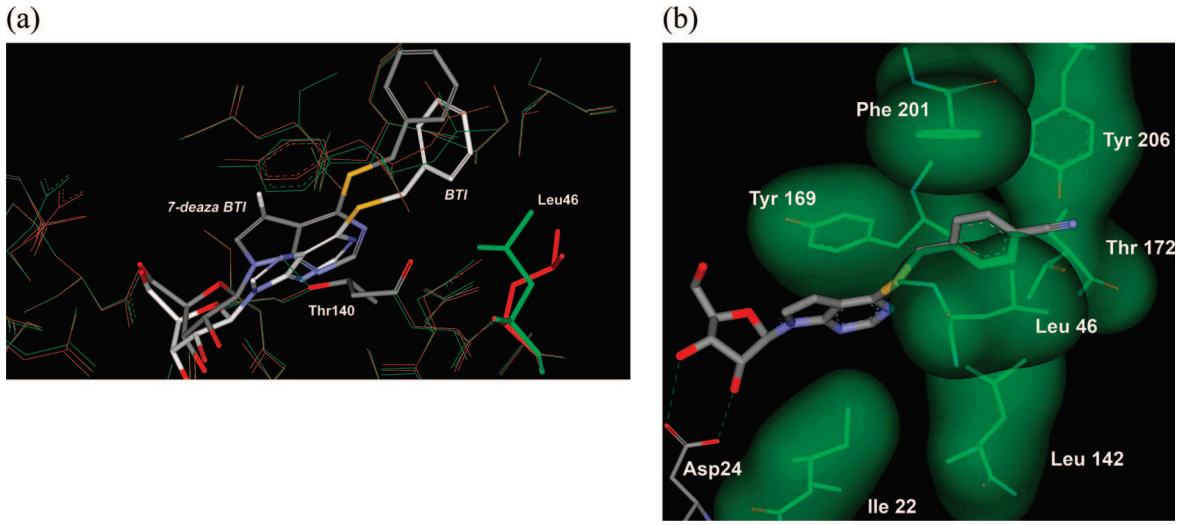


Figure 1. (a) Binding mode of 7-deaza-6-benzylthioinosine (**6a**), showing superposition with 6-benzylthioinosine binding mode. Rigid conformation was observed in 6-benzylthioinosine because of H-bonding with Thr140, and it enforces Leu46 (red) to adopt away-orientation in comparison to Leu46 (green) in the 7-deaza-6-benzylthioinosine complex. (b) Binding mode of 7-deaza-*p*-cyano-6-benzylthioinosine (**6m**), showing significant favorable orientation and position with proper intermolecular interactions (H-bonding with Asp24).

highlighted the molecular basis for the binding of various 6-substituted 9-β-D-ribofuranosylpurines in the active site of *T. gondii* adenosine kinase.[24,27] Our earlier structure−activity relationship and molecular modeling studies[21,24,27] suggested that the position of the ribose ring is mediated by interaction with hydrophilic residues (Asp24, Gly69, and Asn73) while the purine ring orientation is governed by hydrophobic interactions mediated by Tyr169 and Ile22 through a stacking interaction by $\pi-\pi$ and C−H−π, respectively. These studies also demonstrated that substituted benzyl moieties at the 6-position of the purine ring further enhance binding to *T. gondii* adenosine kinase and their potential as antitoxoplasmic agents. Moreover, the degree of binding of these compounds is influenced by the type and position of substituent on the benzyl group. In the present molecular modeling studies we investigated the underlying molecular basis of the effects of eliminating the N7 from 6-benzylthioinosine analogues, as well as the role of substituents at the 7-position and the benzyl group on the binding of these compounds to the active site of *T. gondii* adenosine kinase.

The 7-deaza-6-benzylthioinosine analogues docked successfully into the *T. gondii* adenosine kinase active site by the induced fit method.[24,27] The binding mode showed an anti conformation of the nucleoside with an 2′-endo puckered ribose conformation. The benzylthiol moiety occupied the hydrophobic pocket at the 6-position and was involved in van der Waals interaction with the surrounding residues such as Leu46, Phe201, Tyr206, Thr172, and Leu142. These residues played a crucial role in terms of the binding affinity in response to different atomic substituents on the 6-benzyl group and ultimately defined the binding affinities of these compounds (Table 2).

Molecular modeling studies of the binding mode of 7-deaza-6-benzylthioinosine (**6a**) revealed the absence of H-bonding with Thr140 (Figure 1a) and a lack of movement of the 7-deazapurine ring toward Thr140, which were found to be present in the case of 6-benzylthioinosine binding. The replacement of N7 with C7 in 7-deaza-6-benzylthioinosine (**6a**) caused the loss of the electrostatic interaction between the 7-postion and Thr140, observed in 6-benzylthioinosine binding. The absence of H-bonding with Thr140 allowed flexibility in the movement of the bulky 6-benzylthio group to fit better in the hydrophobic pocket at the 6-position. Therefore, it can be concluded that 7-deaza-6-benzylthioinosine analogues are more appropriate to utilize the hydrophobic pocket at the 6-position for better binding than their 6-benzylthioinosine counterparts. It should also be noted that the introduction of the iodine atom at the C7-position did not improve the binding affinity of 7-iodo-7-deaza-6-benzylthioinosine (**12**). It seems likely that the decrease in the binding affinity of compound **12** is due to poor interaction and ligand-fit profile in the enzyme active site. Binding mode studies suggest that the hydrophobic pockets at the C6 and C7 positions are shared by Leu46. The present studies indicated that the interaction of Leu46 with the iodine atom at the C7-position was not allowed by the presence of the benzylthio group at the C6-position. Hence, the binding affinity of 7-iodo-7-deaza-6-benzylthioinosine (**12**) is decreased when compared with 7-deaza-6-benzylthioinosine (**6a**) (Table 1). These findings were supported further by free energy calculations using the MM-GBSA approach (molecular mechanics energy with an SGB solvation model),[42] which showed significant differences in terms of the strain energy between 7-iodo-7-deaza-6-benzylthioinosine (**12**) and 7-deaza-6-benzylthioinosine (**6a**) (Table 4). Ligand strain energy and receptor strain energy for **12** were higher than those for 7-deaza-6-benzylthioinosine (**6a**) (Table 4), and this clearly suggests that accommodation of 7-iodo-7-deaza-6-benzylthioinosine (**12**) in the active site of *T. gondii* adenosine kinase is relatively unfavorable when compared to that of 7-deaza-6-benzylthioinosine (**6a**).

Table 4. MM-GBSA Free Energy Calculation[42] of 7-Deaza-6-benzylthioinosine Analogues (**6a** and **12**) and 6-Benzylthioinosine

compd	MM-GBSA energy difference results		
	ligand energy in complex	receptor strain energy	total
6a	−38.7	5.8	−53.1
12	−25.06	11.0	−52.7
6-benzylthioinosine	−36.52	7.8	−62.4

The binding affinity of **6a** (27.9 μM) was greatly increased by the addition of the proper substituent on the 6-benzyl group. Figure 1b shows the significant binding mode of 7-deaza-*p*-cyano-6-benzylthioinosine (**6m**), one of the two best ligands (5.2 μM), where the 2′- and 3′-OH formed H-bonding with Asp24 and the *p*-cyanobenzyl group occupied a favorable position and proper orientation in the hydrophobic pocket. Figure

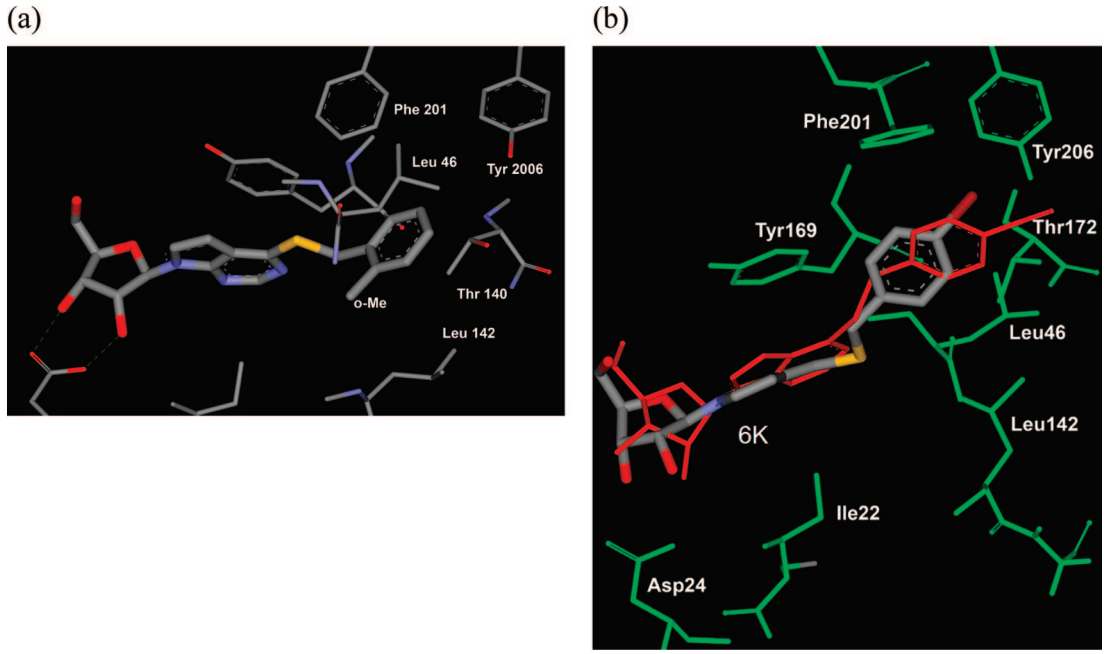


Figure 2. (a) Binding mode of 7-deaza-*o*-methyl-6-benzylthioinosine (**6e**) showing the *o*-methyl orientation, which is away from the hydrophobic pocket and is contributing toward unfavorable binding. (b) Binding mode of 7-deaza-*p*-bromo-6-benzylthioinosine (**6k**) superimposed with 7-deaza-*p*-cyano-6-benzylthioinosine (**6m**, red) showing unfavorable orientation of 7-deazapurine position and 6-benzylthio group of **6k** in the hydrophobic pocket.

Table 5. MBAE (Multiligand Bimolecular Association with Energetics) Calculation of 7-Deaza-6-benzylthioinosine Analogues after Induced Fit Docking and Energy Minimization

		energy difference results (ΔE, kJ/mol)		
compd	K_i [μM]	electrostatic	vdW[a]	total
6a	28 ± 8.6	−244.7	−14.7	−54.2
6e (*o*-CH_3)	228 ± 49.4	−363.8	2.7	−36.6
6k (*p*-Br)	253 ± 56	−399.3	−9.5	−75.5
6m (*p*-CN)	5.2 ± 1.1	−291.9	−53.8	−63.6
6n (*p*-CO_2CH_3)	7.0 ± 1.3	−363.3	−33.9	−70.6
6s (*p*-OCH_3)	5.8 ± 1.0	−414.1	−5.6	−60.1
6w (*o*-Cl-*o*-F)	7.2 ± 1.3	−244.7	−14.7	−84.9

[a] van der Waals interaction.

2 shows the unfavorable orientation of substituted 6-benzylthio group of 7-deaza-*o*-methyl-6-benzylthioinosine (**6e**) and 7-deaza-*p*-bromo-6-benzylthioinosine (**6k**) into the hydrophobic pocket, which resulted in the poor binding of these compounds. The 6-benzylthio group of compound **6k** was oriented away from the hydrophobic pocket, and the overall conformation of **6k** was unsuitable in terms of its position in the active site in comparison with **6m** as shown in Figure 2b. The van der Waals binding energy difference of **6k** (−9.5 kJ/mol) was very low in comparison to **6m** (−53.8 kJ/mol), which shows that unfavorable interactions were mediated by the *p*-bromobenzyl group in the hydrophobic pocket. Energetic correlations of a few representative compounds are shown in Table 5 and reveal a qualitative comparison with respect to experimental data. Table 5 clearly shows the favorable binding energy differences of **6m**, **6n**, **6s**, and **6w** and the relatively poor binding energy of **6a** and **6e**. These molecular modeling studies suggest that the proper substitution on the 6-benzylthio group renders the compound active or inactive because of its conformational orientation and position in the 6-position hydrophobic pocket. In addition, proper substitution on the 6-benzylthio group affects the overall conformation of the analogue to fit into the active site of *T. gondii* adenosine kinase.

In summary, 7-deaza-6-benzylthioinosine analogues were synthesized and evaluated as ligands of *T. gondii* adenosine kinase. 7-Deaza-*p*-cyano-6-benzylthioinosine (**6m**) and 7-deaza-*p*-methoxy-6-benzylthioinosine (**6s**) were the best ligands and exhibited better binding affinity than the natural substrate adenosine. Structure−activity relationships indicated that the presence of a nitrogen at the 7-position is not an essential structural requirement for binding, since 7-deaza-6-benzylthioinosine analogues have higher binding affinities than their corresponding 6-benzylthioinosine counterparts. Molecular modeling demonstrated that 7-deaza-6-benzylthioinosine analogues do not form H-bonding with Thr140 which provides flexibility in the movement of the benzylthio group for better fitting in the 6-position hydrophobic pocket. Furthermore, 7-deaza-6-benzylthioinosine analogues with certain substituents on the benzyl group may better utilize the 6-position hydrophobic pocket for binding. Ligands with high affinity to the enzyme, **6m** and **6s**, displayed potent antitoxoplasmic activity in cell culture with IC_{50} values of 5.3 and 4.6 μM, respectively, without significant host cell toxicity. The present studies provide important insights into the structual basis for substrate specificity of *T. gondii* adenosine kinase and provide direction for further development of potent and less cytotoxic antitoxoplasmic agents.

Experimental Section

General. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. Optical rotation was determined on a Jasco DIP-370 digital polarimeter. UV spectra were obtained on a Beckman DU-650 spectrophotometer. NMR spectra were recorded on a Varian Inova 500 spectrometer, and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane as internal reference. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quarter), or m (multiplet). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Silica gel 60 (220−440 mesh) was used for flash column chromatography. 6-Chloro-7-deazapurine was purchased from Toronto Research Chemicals, Inc. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

6-Hydroxymethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (2). A solution of D-ribose (40.0 g, 0.266 mol) in acetone (133.0 mL) was treated with *p*-toluenesulfonic acid (1.90 g, 13.3 mmol)

and 2,2-dimethoxypropane (49.0 mL, 400 mol). The reaction mixture was stirred for 1 h at room temperature and then neutralized with solid $NaHCO_3$ (1.11 g, 13.3 mmol). The suspension was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3:1 to 1:1) to give anomeric mixture **2** (41.0 g, 81%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) for β-isomer δ 1.33 (s, 3H), 1.49 (s, 3H), 3.54 (m, 1H), 3.73 (m, 1H), 3.75 (d, J = 2.0 Hz, 1H), 4.42 (m, 1H), 4.59 (d, J = 6.5 Hz, 1H), 4.68 (m, 1H), 4.85 (d, J = 6.0 Hz, 1H), 5.43 (m, 1H); for α-isomer (diagnostic peaks only) δ 1.40 (s, 3H), 1.58 (s, 3H), 4.06 (m, 1H), 4.19 (m, 1H), 4.64 (m, 1H), 4.75 (m, 1H), 3.78 (d, J = 2.0 Hz, 1H), 3.71 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) for β-isomer δ 24.7, 26.3, 63.4, 81.6, 86.7, 87.6, 102.7, 112.2.

5-*O*-[(1,1-Dimethylethyl)dimethysilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranose (3). A solution of compound **2** (8.60 g, 45.2 mmol) in anhydrous CH_2Cl_2 (150.7 mL) was treated with *tert*-butyldimethylsilyl chloride (8.86 g, 58.8 mmol) and imidazole (9.24 g, 131 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and kept for 2 h. The reaction mixture was washed with water. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 7:1) to give **3** (11.4 g, 83%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) for β-isomer δ 0.00 (s, 6H), 0.79 (s, 9H), 1.19 (s, 3H), 1.35 (s, 3H), 3.62 (m, 2H), 4.21 (m, 1H), 4.37 (d, J = 5.5 Hz, 1H), 4.56 (d, J = 5.5 Hz, 1H), 4.65 (d, J = 11.0 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H); for α-isomer (diagnostic peaks only) δ −0.08 (s, 6H), 0.75 (s, 9H), 1.25 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for β-isomer δ −5.7, 18.2, 24.8, 25.7, 26.4, 64.7, 81.7, 86.9, 87.5, 103.4, 111.9.

4-Chloro-7-[5-*O*-[(1,1-dimethylethyl)dimethysilyl]-2,3-*O*-(1-methylethylidene)-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (4). A mixture of compound **3** (4.08 g, 13.4 mmol) and CCl_4 (1.7 mL, 17.4 mmol) in anhydrous THF (67.0 mL) was treated with hexamethylphosphoramide (3.2 mL, 17.4 mmol) at −78 °C. The reaction mixture was allowed to warm up at −30 °C and stirred for 1 h. This resulting solution of chloro sugar was cannulated into a well-stirred mixture of 6-chloro-7-deazapurine (1.37 g, 8.93 mmol), KOH (1.00 g, 17.9 mmol), and tris[2-(2-methoxyethoxy-)ethyl]amine (2.9 mL, 8.93 mmol) in anhydrous CH_3CN (55.8 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and kept for 16 h. After the solvent was removed in vacuo, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 95:5 to 9:1) to give anomeric mixture of **4** (1.57 g, 40% for two steps) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) for β-isomer δ 0.07 (s, 6H), 0.90 (s, 9H), 1.39 (s, 3H), 1.65 (s, 3H), 3.80 (dd, J_1 = 11.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.5 Hz, 1H), 4.36 (q, J = 3.2 Hz, 1H), 4.95 (m, 1H), 5.06 (m, 1H), 6.41 (d, J = 3.0 Hz, 1H), 6.63 (d, J = 3.5 Hz, 1H), 7.57 (d, J = 3.5 Hz, 1H), 8.66 (s, 1H); for α-isomer (diagnostic peaks only) δ 6.55 (d, J = 3.5 Hz, 1H, H-7), 6.84 (d, J = 4.0 Hz, 1H, H-1′), 7.56 (d, J = 4.0 Hz, 1H, H-8), 8.53 (s, 1H, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) for β-isomer δ −5.4, 18.3, 25.4, 25.8, 27.3, 63.4, 80.8, 85.0, 86.1, 90.6, 100.4, 114.2, 118.2, 127.1, 150.9, 152.1; HRMS $[M + H]^+$ *m*/*z* calcd for $C_{20}H_{31}ClN_3O_4Si$ 440.1772, found 440.1739.

4-Chloro-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (5a) and α-Anomer (5b). A mixture of compound **4** (1.57 g, 3.57 mmol) and Dowex 50WX8-200 (0.785 g) in anhydrous MeOH (36.0 mL) was stirred for 8 h at room temperature. The resin was filtered off, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give β-anomer **5a** (0.881 g, 86%) and α-anomer **5b** (0.088 g, 9%) as white solids. **5a**: mp 160−161 °C [lit.[35] mp 160−162 °C]; $[\alpha]_D^{27}$ −46.17 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 274.0 nm [lit.[35] UV (MeOH) λ_{max} 273.0 nm]; ^{1}H NMR (500 MHz, CD_3OD) δ 3.80 (dd, J_1 = 12.0 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (q, J = 3.3 Hz, 1H), 4.35 (m, 1H), 4.62 (t, J = 5.8 Hz, 1H), 6.31 (d, J = 5.5 Hz, 1H), 6.75 (d, J = 4.0 Hz, 1H), 7.90 (d, J = 4.0 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 61.8, 70.9, 74.6, 85.6, 89.0, 99.7, 118.4, 128.6, 150.0, 151.2, 151.5; HRMS $[M + H]^+$ *m*/*z* calcd for $C_{11}H_{13}ClN_3O_4$ 286.0594, found 286.0594. **5b**: UV (MeOH) λ_{max} 273.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 3.71 (dd, J_1 = 12.5 Hz, J_2 = 4.0 Hz, 1H), 3.86 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.31 (m, 1H), 4.37 (d, J = 5.8 Hz, 1H), 4.50 (t, J = 5.0 Hz, 1H), 6.69 (d, J = 4.0 Hz, 1H), 6.75 (d, J = 5.0 Hz, 1H), 7.97 (d, J = 4.0 Hz, 1H), 8.59 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 61.6, 70.9, 71.6, 84.6, 85.0, 98.4, 117.7, 130.7, 149.7, 150.9, 151.2; HRMS $[M + H]^+$ *m*/*z* calcd for $C_{11}H_{13}ClN_3O_4$ 286.0594, found 286.0602.

General Procedure for the Compounds 6a−k,m−p,r,s,u−w. To a solution of compound **5a** (65.0 mg, 0.228 mmol) in anhydrous THF (5.7 mL) were added the appropriate benzylthiol (0.912 mmol) and sodium hydride (0.456 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and kept for 1 h. Excess hydride was quenched by addition of methanol, and then the reaction mixture was concentrated in vacuo. The residues were purified by short column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give the desired products as white solids.

4-Benzylthio-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6a). Yield 85%; mp 145−147 °C; $[\alpha]_D^{27}$ −64.33 (*c* 0.21, EtOH); UV (H_2O) λ_{max} 299.0 nm (ε 11 908, pH 2), 296.0 nm (ε 13 827, pH 7), 296.0 nm (ε 13 841, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (d, J = 12.5 Hz, 1H), 3.89 (d, J = 9.5 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (m, 1H), 4.68 (s, 2H), 6.18 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 4.0 Hz, 1H), 7.27 (m, 1H), 7.32 (m, 2H), 7.47 (d, J = 7.0 Hz, 2H), 7.63 (d, J = 3.5 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 32.3, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 116.9, 126.5, 126.9, 128.2, 128.7, 137.8, 148.0, 149.8, 161.2. Anal. ($C_{18}H_{19}N_3O_4S \cdot 0.3H_2O$) C, H, N, S.

4-[(2-Fluorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6b). Yield 82%; mp 135−136 °C; $[\alpha]_D^{25}$ −76.88 (*c* 0.16, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 13 079, pH 2), 296.0 nm (ε 14 749, pH 7), 296.0 nm (ε 13 925, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.65 (t, J = 5.8 Hz, 1H), 4.71 (s, 2H), 6.18 (d, J = 5.5 Hz, 1H), 6.57 (d, J = 4.0 Hz, 1H), 7.12 (m, 2H), 7.30 (m, 1H), 7.58 (m, 1H), 7.63 (d, J = 3.5 Hz, 1H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 25.5, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 114.9 (d, J = 21.5 Hz), 117.0, 124.0, 124.9 (d, J = 14.9 Hz), 126.5, 129.0 (d, J = 8.0 Hz), 131.1, 148.1, 149.8, 160.6, 161.1 (d, J = 244.6 Hz). Anal. ($C_{18}H_{18}FN_3O_4S$) C, H, N, S.

4-[(2-Chlorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6c). Yield 83%; mp 146−147 °C; $[\alpha]_D^{25}$ −75.55 (*c* 0.22, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 13 470, pH 2), 296.0 nm (ε 14 815, pH 7), 296.0 nm (ε 14 193, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.66 (t, J = 6.0 Hz, 1H), 4.81 (s, 2H), 6.19 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 3.5 Hz, 1H), 7.27 (m, 2H), 7.45 (m, 1H), 7.63 (d, J = 4.0 Hz, 1H), 7.67 (m, 1H), 8.66 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 30.0, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 117.0, 126.5, 126.8, 128.7, 129.2, 131.1, 134.0, 135.5, 148.1, 149.8, 160.7. Anal. ($C_{18}H_{18}ClN_3O_4S$) C, H, N, S.

4-[(2-Bromobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6d). Yield 86%; mp 158−160 °C; $[\alpha]_D^{25}$ −56.23 (*c* 0.29, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 13 619, pH 2), 296.0 nm (ε 14 390, pH 7), 296.0 nm (ε 14 305, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.65 (t, J = 6.0 Hz, 1H), 4.80 (s, 2H), 6.18 (d, J = 6.0 Hz, 1H), 6.56 (d, J = 3.0 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.62 (m, 2H), 7.67 (d, J = 7.5 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 32.7, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 116.9, 124.3, 126.5, 127.4, 128.9, 131.1, 132.6, 137.2, 148.0, 149.8, 160.6. Anal. ($C_{18}H_{18}BrN_3O_4S$) C, H, N, S.

4-[(2-Methylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6e). Yield 83%; mp 136−137 °C; $[\alpha]_D^{28}$ −75.57 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 298.0 nm (ε 14 757, pH 2), 297.0

nm (ε 15 868, pH 7), 297.0 nm (ε 15 558, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.46 (s, 3H), 3.79 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (m, 1H), 4.34 (m, 1H), 4.66 (t, J = 4.8 Hz, 1H), 4.71 (s, 2H), 6.19 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 4.0 Hz, 1H), 7.19 (m, 3H), 7.44 (d, J = 7.5 Hz, 1H), 7.63 (m, 1H), 8.65 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 18.0, 30.8, 62.1, 71.1, 74.3, 85.8, 89.4, 99.1, 117.0, 125.8, 126.4, 127.4, 129.7, 130.1, 134.9, 136.8, 148.1, 149.9, 161.4. Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N, S.

4-[(3-Nitrobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6f). Yield 72%; mp 197−199 °C; $[\alpha]_D^{25}$ −54.88 (*c* 0.09, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 18 159, pH 2), 292.0 nm (ε 18 677, pH 7), 292.0 nm (ε 18 299, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.32 (m, 1H), 4.64 (t, J = 5.8 Hz, 1H), 4.80 (s, 2H), 6.19 (d, J = 6.0 Hz, 1H), 6.60 (d, J = 3.5 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.65 (d, J = 4.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 8.13 (m, 1H), 8.42 (s, 1H), 8.65 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.1, 62.0, 71.1, 74.3, 85.7, 89.3, 98.9, 117.0, 121.7, 123.5, 126.7, 129.3, 135.1, 141.1, 148.2, 149.9, 159.9. Anal. ($C_{18}H_{18}N_4O_6S$) C, H, N, S.

4-[(3-Trifluoromethylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6g). Yield 82%; mp 161−163 °C; $[\alpha]_D^{25}$ −65.27 (*c* 0.19, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 13 117, pH 2), 296.0 nm (ε 14 319, pH 7), 296.0 nm (ε 14 291, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (t, J = 5.5 Hz, 1H), 4.75 (s, 2H), 6.19 (d, J = 6.0 Hz, 1H), 6.59 (d, J = 3.5 Hz, 1H), 7.51 (m, 1H), 7.56 (m, 1H), 7.64 (m, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.82 (m, 1H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.5, 62.0, 71.1, 74.3, 85.7, 89.3, 99.0, 117.0, 123.5, 125.4, 126.6, 128.9, 129.3, 130.3 (q, J = 32.0 Hz), 132.5, 139.9, 148.1, 149.8, 160.3. Anal. ($C_{19}H_{18}F_3N_3O_4S\cdot0.1H_2O$) C, H, N, S.

4-[(3-Methylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6h). Yield 85%; mp 149−150 °C; $[\alpha]_D^{25}$ −58.12 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 299.0 nm (ε 14 105, pH 2), 297.0 nm (ε 15 211, pH 7), 297.0 nm (ε 14 638, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.34 (s, 3H), 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.65 (m, 3H), 4.75 (s, 2H), 6.18 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 3.5 Hz, 1H), 7.09 (d, J = 7.5 Hz, 1H), 7.20 (m, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.29 (m, 1H), 7.63 (d, J = 4.0 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.0, 32.4, 62.0, 71.1, 74.3, 85.8, 89.4, 99.1, 116.9, 125.8, 126.4, 127.6, 128.1, 129.4, 137.5, 138.0, 148.0, 149.8, 161.3. Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N, S.

4-[(4-Fluorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6i). Yield 83%; mp 99−101 °C; $[\alpha]_D^{25}$ −67.30 (*c* 0.21, MeOH); UV (H_2O) λ_{max} 298.0 nm (ε 12 936, pH 2), 296.0 nm (ε 14 171, pH 7), 296.0 nm (ε 14 135, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.63 (s, 2H), 4.66 (t, J = 6.0 Hz, 1H), 6.18 (d, J = 5.5 Hz, 1H), 6.55 (d, J = 3.5 Hz, 1H), 7.03 (m, 2H), 7.48 (m, 2H), 7.61 (d, J = 3.5 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.5, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 114.8 (d, J = 21.9 Hz), 116.9, 126.5, 130.6 (d, J = 8.1 Hz), 134.0, 148.0, 149.8, 160.9, 162.0 (d, J = 242.8 Hz). Anal. ($C_{18}H_{18}FN_3O_4S$) C, H, N, S.

4-[(4-Chlorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6j). Yield 81%; mp 162−163 °C; $[\alpha]_D^{28}$ −76.24 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 298.0 nm (ε 15 494, pH 2), 296.0 nm (ε 16 401, pH 7), 296.0 nm (ε 16 312, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.65 (m, 3H), 6.19 (d, J = 5.5 Hz, 1H), 6.58 (d, J = 4.0 Hz, 1H), 7.32 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 4.0 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.4, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 117.0, 126.5, 128.2, 130.3, 132.6, 137.0, 148.1, 149.9, 160.7. Anal. ($C_{18}H_{18}ClN_3O_4S$) C, H, N, S.

4-[(4-Bromobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6k). Yield 80%; mp 159−161 °C; $[\alpha]_D^{25}$ −57.64 (*c* 0.16, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 14 589, pH 2), 296.0 nm (ε 15 973, pH 7), 296.0 nm (ε 15 930, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (m, 3H), 6.18 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 3.5 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 4.0 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.4, 62.0, 71.1, 74.3, 85.7, 89.3, 99.0, 117.0, 120.6, 126.6, 130.7, 131.2, 137.5, 148.1, 149.8, 160.6. Anal. ($C_{18}H_{18}BrN_3O_4S$) C, H, N, S.

4-[(4-Cyanobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6m). Yield 77%; mp 184−186 °C; $[\alpha]_D^{25}$ −61.99 (*c* 0.15, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 14 656, pH 2), 296.0 nm (ε 15 474, pH 7), 296.0 nm (ε 14 860, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.64 (t, J = 5.5 Hz, 1H), 4.74 (s, 2H), 6.19 (d, J = 6.0 Hz, 1H), 6.58 (d, J = 4.0 Hz, 1H), 7.65 (d, J = 4.0 Hz, 1H), 7.68 (m, 4H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.5, 62.0, 71.1, 74.3, 85.7, 89.3, 98.9, 110.5, 117.0, 118.3, 126.7, 129.7, 131.9, 144.5, 148.2, 149.8, 160.0. Anal. ($C_{19}H_{18}N_4O_4S$) C, H, N, S.

4-[(4-Methoxycarbonylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6n). Yield 79%; mp 168−170 °C; $[\alpha]_D^{25}$ −55.59 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 13 962, pH 2), 296.0 nm (ε 15 088, pH 7), 296.0 nm (ε 14 616, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.91 (m, 4H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (t, J = 5.5 Hz, 1H), 4.74 (s, 2H), 6.19 (d, J = 5.5 Hz, 1H), 6.59 (d, J = 3.5 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 3.5 Hz, 1H), 7.98 (d, J = 7.5 Hz, 2H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.7, 51.2, 62.0, 71.1, 74.3, 85.7, 89.3, 99.0, 117.0, 126.6, 128.9, 129.3, 144.0, 148.1, 149.8, 160.4, 166.9, 180.1. Anal. ($C_{20}H_{21}N_3O_6S$) C, H, N, S.

4-[(4-Methylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6o). Yield 82%; mp 123−124 °C; $[\alpha]_D^{25}$ −66.72 (*c* 0.22, MeOH); UV (H_2O) λ_{max} 299.0 nm (ε 14 362, pH 2), 297.0 nm (ε 16 169, pH 7), 297.0 nm (ε 16 026, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.33 (s, 3H), 3.78 (dd, J_1 = 13.0 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.63 (s, 2H), 4.66 (t, J = 6.0 Hz, 1H), 6.18 (d, J = 5.5 Hz, 1H), 6.57 (d, J = 3.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 2H), 7.35 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 4.0 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 19.7, 32.2, 62.1, 71.1, 74.3, 85.7, 89.4, 99.1, 117.0, 126.4, 128.6, 128.8, 134.5, 136.7, 148.1, 149.9, 161.4. Anal. ($C_{19}H_{21}N_3O_4S\cdot0.1H_2O$) C, H, N, S.

4-[(4-*tert*-Butylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6p). Yield 81%; mp 83−85 °C; $[\alpha]_D^{25}$ −51.07 (*c* 0.23, MeOH); UV (H_2O) λ_{max} 299.0 nm (ε 12 456, pH 2), 297.0 nm (ε 11 421, pH 7), 297.0 nm (ε 12 420, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 1.33 (s, 9H), 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.5 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.34 (m, 1H), 4.63 (s, 2H), 4.66 (t, J = 5.8 Hz, 1H), 6.18 (d, J = 6.0 Hz, 1H), 6.57 (d, J = 4.0 Hz, 1H), 7.37 (m, 4H), 7.62 (d, J = 4.0 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 30.4, 32.1, 33.9, 62.1, 71.1, 74.3, 85.7, 89.5, 99.1, 117.0, 125.1, 126.4, 128.4, 134.5, 148.1, 149.9, 150.0, 161.4. Anal. ($C_{22}H_{27}N_3O_4S\cdot0.1H_2O$) C, H, N, S.

4-[(4-Trifluoromethoxybenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6r). Yield 79%; mp 159−160 °C; $[\alpha]_D^{28}$ −57.12 (*c* 0.23, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 11 500, pH 2), 296.0 nm (ε 14 378, pH 7), 296.0 nm (ε 13 504, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (t, J = 5.8 Hz, 1H), 4.71 (s, 2H), 6.19 (d, J = 5.5 Hz, 1H), 6.59 (d, J = 3.5 Hz, 1H), 7.23 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 3.5 Hz, 1H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.3, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 117.0, 120.5 (q, J = 254.0 Hz), 120.6, 126.5, 130.4, 137.5, 148.1, 148.2, 149.9, 160.6. Anal. ($C_{19}H_{18}F_3N_3O_5S$) C, H, N, S.

4-[(4-Methoxybenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6s). Yield 86%; mp 163−164 °C; $[\alpha]_D^{29}$ −68.73 (*c* 0.13, MeOH); UV (H_2O) λ_{max} 299.0 nm (ε 14 510, pH 2), 297.0 nm (ε 16 537, pH 7), 298.0 nm (ε 15 818, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (m, 4H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.62 (s, 2H), 4.66 (t, *J* = 6.0 Hz, 1H), 6.18 (d, *J* = 6.0 Hz, 1H), 6.57 (d, *J* = 3.5 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 4.0 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 32.0, 54.3, 62.1, 71.1, 74.3, 85.7, 89.4, 99.1, 113.6, 117.0, 126.4, 129.5, 129.9, 148.1, 149.9, 159.1, 161.4. Anal. ($C_{19}H_{21}N_3O_5S$) C, H, N, S.

4-[(2,4-Dichlorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6u). Yield 80%; mp 160−161 °C; $[\alpha]_D^{25}$ −48.77 (*c* 0.22, MeOH); UV (H_2O) λ_{max} 296.0 nm (ε 8008, pH 2), 295.0 nm (ε 10121, pH 7), 296.0 nm (ε 13 509, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (t, *J* = 5.8 Hz, 1H), 4.77 (s, 2H), 6.19 (d, *J* = 6.0 Hz, 1H), 6.57 (d, *J* = 4.0 Hz, 1H), 7.28 (dd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.63 (d, *J* = 4.0 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 29.4, 62.0, 71.1, 74.3, 85.7, 89.4, 98.9, 117.0, 126.6, 127.0, 128.8, 132.1, 133.5, 134.7, 134.8, 148.2, 149.8, 160.2. Anal. ($C_{18}H_{17}Cl_2N_3O_4S$) C, H, N, S.

4-[(3,4-Dichlorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6v). Yield 79%; mp 133−134 °C; $[\alpha]_D^{26}$ −78.24 (*c* 0.11, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 14 268, pH 2), 296.0 nm (ε 14 890, pH 7), 296.0 nm (ε 14 495, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.77 (dd, J_1 = 12.5 Hz, J_2 = 3.5 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (m, 3H), 6.19 (d, *J* = 5.5 Hz, 1H), 6.59 (d, *J* = 4.0 Hz, 1H), 7.45 (m, 2H), 7.65 (d, *J* = 4.0 Hz, 1H), 7.67 (d, *J* = 1.5 Hz, 1H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 30.8, 62.0, 71.1, 74.3, 85.7, 89.3, 99.0, 117.0, 126.6, 128.6, 130.1, 130.6, 130.7, 131.7, 139.4, 148.1, 149.8, 160.1. Anal. ($C_{18}H_{17}Cl_2N_3O_4S$) C, H, N, S.

4-[(2-Chloro-6-fluorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6w). Yield 84%; mp 170−172 °C; $[\alpha]_D^{26}$ −72.80 (*c* 0.20, MeOH); UV (H_2O) λ_{max} 296.0 nm (ε 14 071, pH 2), 296.0 nm (ε 15 191, pH 7), 296.0 nm (ε 14 707, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.79 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.34 (m, 1H), 4.66 (t, *J* = 5.8 Hz, 1H), 4.90 (s, 2H), 6.20 (d, *J* = 6.5 Hz, 1H), 6.57 (d, *J* = 3.5 Hz, 1H), 7.16 (m, 1H), 7.36 (m, 2H), 7.64 (d, *J* = 3.0 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 23.8, 62.0, 71.1, 74.3, 85.7, 89.3, 98.9, 114.0 (d, *J* = 22.4 Hz), 116.8, 123.0 (d, *J* = 18.1 Hz), 125.3, 126.6, 129.7 (d, *J* = 10.0 Hz), 135.5, 148.1, 149.9, 160.6, 161.5 (d, *J* = 248.5 Hz). Anal. ($C_{18}H_{17}ClFN_3O_4S$) C, H, N, S.

General Procedure for the Compounds (6l, 6q, and 6t). To a solution of compound **7** (50.0 mg, 0.176 mmol) in H_2O (2.2 mL) were added concentrated NH_4OH (0.03 mL) and the appropriate benzyl halide (0.221 mmol). The reaction mixture was stirred for 11 h at room temperature. The resulting precipitates were filtered and purified by short column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give the desired products as white solids.

4-[(4-Nitrobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6l). Yield 86%; mp 163−164 °C; $[\alpha]_D^{25}$ −71.18 (*c* 0.15, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 21 370, pH 2), 293.0 nm (ε 22 011, pH 7), 293.0 nm (ε 21 278, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.0 Hz, J_2 = 3.5 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.32 (m, 1H), 4.64 (t, *J* = 5.8 Hz, 1H), 4.80 (s, 2H), 6.19 (d, *J* = 5.5 Hz, 1H), 6.59 (d, *J* = 3.5 Hz, 1H), 7.65 (d, *J* = 3.5 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 2H), 8.20 (d, *J* = 8.5 Hz, 2H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 31.2, 62.0, 71.1, 74.3, 85.7, 89.3, 98.9, 117.0, 123.1, 126.7, 129.8, 146.5, 147.0, 148.2, 149.9, 159.9. Anal. ($C_{18}H_{18}N_4O_6S$) C, H, N, S.

4-[(4-Vinylbenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6q). Yield 92%; mp 175−176 °C; $[\alpha]_D^{25}$ −69.81 (*c* 0.16, MeOH); UV (H_2O) λ_{max} 297.0 nm (ε 15 766, pH 2), 296.0 nm (ε 17 382, pH 7), 296.0 nm (ε 17 169, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H), 3.89 (dd, J_1 = 12.0 Hz, J_2 = 2.5 Hz, 1H), 4.15 (m, 1H), 4.33 (m, 1H), 4.65 (m, 3H), 5.23 (d, *J* = 10.5 Hz, 1H), 5.78 (d, *J* = 17.5 Hz, 1H), 6.18 (d, *J* = 6.0 Hz, 1H), 6.58 (d, *J* = 3.5 Hz, 1H), 6.73 (dd, J_1 = 17.5 Hz, J_2 = 10.5 Hz, 1H), 7.41 (m, 4H), 7.63 (d, *J* = 3.5 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 32.1, 62.0, 71.1, 74.3, 85.7, 89.4, 99.0, 112.6, 117.0, 126.0, 126.5, 128.9, 136.4, 136.7, 137.4, 148.0, 149.8, 161.1. Anal. ($C_{20}H_{21}N_3O_4S$) C, H, N, S.

4-[(2,4-Difluorobenzyl)thio]-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6t). Yield 95%; mp 143−144 °C; $[\alpha]_D^{25}$ −56.75 (*c* 0.24, MeOH); UV (H_2O) λ_{max} 296.0 nm (ε 11 824, pH 2), 295.0 nm (ε 12 186, pH 7), 296.0 nm (ε 11 637, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (dd, J_1 = 13.0 Hz, J_2 = 3.0 Hz, 1H), 3.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 4.14 (m, 1H), 4.33 (m, 1H), 4.65 (t, *J* = 5.8 Hz, 1H), 4.69 (s, 2H), 6.18 (d, *J* = 6.0 Hz, 1H), 6.57 (d, *J* = 4.0 Hz, 1H), 6.91 (m, 1H), 6.98 (m, 1H), 7.63 (m, 2H), 8.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 24.9, 62.0, 71.1, 74.3, 85.7, 89.3, 98.9, 103.2 (t, *J* = 25.8 Hz), 110.8 (dd, J_1 = 21.5 Hz, J_2 = 3.8 Hz), 117.0, 121.3 (dd, J_1 = 14.7 Hz, J_2 = 3.9 Hz), 126.6, 132.1 (dd, J_1 = 10.2 Hz, J_2 = 5.1 Hz), 148.1, 149.8, 160.4, 161.1 (dd, J_1 = 247.6 Hz, J_2 = 12.4 Hz), 162.5 (dd, J_1 = 246.1 Hz, J_2 = 11.4 Hz). Anal. ($C_{18}H_{17}F_2N_3O_4S$) C, H, N, S.

7-(β-D-Ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-thione (7). To a solution of compound **5a** (160 mg, 0.560 mmol) in anhydrous EtOH (5.6 mL) was added thiourea (86.0 mg, 1.12 mmol). The reaction mixture was heated to reflux for 3 h. After the solvent was removed in vacuo, the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give **7** (135 mg, 85%) as a white solid: mp 208−209 °C; $[\alpha]_D^{28}$ −85.30 (*c* 0.47, MeOH); UV (H_2O) λ_{max} 324.0 nm (ε 22 197, pH 2), 324.0 nm (ε 21 362, pH 7), 310.0 nm (ε 20 225, pH 11); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.56 (m, 1H), 3.64 (m, 1H), 3.92 (m, 1H), 4.10 (m, 1H), 4.33 (m, 1H), 5.06 (t, *J* = 5.3 Hz, 1H), 5.19 (d, *J* = 4.5 Hz, 1H), 5.40 (d, *J* = 6.0 Hz, 1H), 6.07 (d, *J* = 6.0 Hz, 1H), 6.72 (d, *J* = 3.5 Hz, 1H), 7.64 (d, *J* = 4.0 Hz, 1H), 8.14 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 61.9, 71.0, 74.9, 85.7, 87.3, 105.2, 121.0, 124.6, 143.8, 177.0. Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N, S.

5-Iodo-4-chloropyrrolo[2,3-*d*]pyrimidine (9). 6-Chloro-7-deazapurine **8** (0.430 g, 2.80 mmol) was treated with *N*-iodosuccinimide (0.630 g, 2.80 mmol) in DMF (9.0 mL). The reaction mixture was stirred for 2 h at room temperature. After the solvent was removed in vacuo, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3:1) to give **9** (0.728 g, 93%) as a white solid: mp 197−199 °C; UV (MeOH) λ_{max} 305.0 nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (s, 1H), 8.61 (s, 1H), 12.98 (br, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.1, 116.3, 134.3, 150.9, 151.2, 152.0.

4-Chloro-5-iodo-7-[5-*O*-[(1,1-dimethylethyl)dimethysilyl]-2,3-*O*-(1-methylethylidene)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (10). A mixture of compound **3** (0.200 g, 0.657 mmol) and CCl_4 (70 μL, 0.723 mmol) in anhydrous THF (6.0 mL) was treated with hexamethylphosphoramide (0.13 mL, 0.723 mmol) at −78 °C. The reaction mixture was allowed to warm up at −30 °C and stirred for 1 h. This resulting solution of chloro sugar was cannulated into a well-stirred mixture of compound **9** (0.141 g, 0.505 mmol), KOH (57.0 mg, 1.01 mmol), and tris[2-(2-methoxyethoxy)ethyl]amine (0.19 mL, 0.607 mmol) in anhydrous CH_3CN (6.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and kept for 16 h. After the solvent was removed in vacuo, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 9:1) to give **10** (126 mg, 44% for two steps) as a colorless oil: $[\alpha]_D^{28}$ −66.34 (*c* 1.8, $CHCl_3$); UV (MeOH) λ_{max} 305.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.90 (s, 9H), 1.36 (s, 3H), 1.63 (s, 3H), 3.79 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 3.90 (dd, J_1 = 11.5 Hz, J_2 = 3.0 Hz, 1H),

4.39 (m, 1H), 4.88 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.0$ Hz, 1H), 4.92 (dd, $J_1 = 6.0$ Hz, $J_2 = 3.0$ Hz, 1H), 6.41 (d, $J = 3.0$ Hz, 1H), 7.76 (s, 1H), 8.63 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.2, 18.5, 25.4, 26.0, 27.4, 52.3, 63.6, 80.9, 85.5, 86.3, 90.9, 114.2, 117.4, 132.1, 150.5, 151.1, 152.6; HRMS $[M + H]^+$ *m/z* calcd for $C_{20}H_{30}ClIN_3O_4Si$ 566.0739, found 566.0727.

4-Chloro-5-iodo-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (11). Compound **11** was prepared from compound **10** (94.5 mg, 0.167 mmol) under the procedure outlined above for compound **5** and was obtained as a white solid (64 mg, 93%): mp 192−194 °C; $[\alpha]_D^{23}$ −55.18 (*c* 0.32, MeOH); UV (MeOH) λ_{max} 306.0 nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.58 (m, 1H), 3.67 (m, 1H), 3.95 (m, 1H), 4.12 (m, 1H), 4.40 (q, $J = 5.5$ Hz, 1H), 5.14 (t, $J = 5.0$ Hz, 1H), 5.24 (d, $J = 5.0$ Hz, 1H), 5.47 (d, $J = 6.0$ Hz, 1H), 6.21 (d, $J = 6.5$ Hz, 1H), 8.27 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 54.1, 61.7, 70.8, 74.8, 86.0, 87.5, 117.1, 134.0, 151.2, 151.5, 151.6; HRMS $[M + H]^+$ *m/z* calcd for $C_{11}H_{12}ClIN_3O_4$ 411.9561, found 411.9567.

4-Benzylthio-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12). A solution of compound **11** (55.0 mg, 0.134 mmol) in anhydrous THF (4.5 mL) was treated with benzylthiol (63 μL, 0.536 mmol) and sodium hydride (6.43 mg, 0.268 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Excess hydride was quenched by addition of methanol, and then the reaction mixture was concentrated to dryness in vacuo. The residues were purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give **12** (56 mg, 84%) as a white solid: mp 223−224 °C; $[\alpha]_D^{25}$ −56.85 (*c* 0.33, DMSO); UV (H_2O) λ_{max} 312.0 nm (ε 6106, pH 2), 312.0 nm (ε 5534, pH 7), 312.0 nm (ε 8631, pH 11); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.56 (m, 1H), 3.65 (m, 1H), 3.92 (m, 1H), 4.10 (m, 1H), 4.37 (q, $J = 5.5$ Hz, 1H), 4.65 (m, 2H), 5.11 (t, $J = 5.0$ Hz, 1H), 5.20 (d, $J = 5.0$ Hz, 1H), 5.42 (d, $J = 6.5$ Hz, 1H), 6.17 (d, $J = 6.0$ Hz, 1H), 7.28 (m, 1H), 7.35 (m, 2H), 7.49 (m, 2H), 8.27 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 32.9, 53.3, 61.8, 70.9, 74.7, 85.9, 87.2, 117.2, 127.7, 129.0, 129.6, 131.4, 138.0, 149.3, 151.0, 161.1; HRMS $[M + H]^+$ *m/z* calcd for $C_{18}H_{19}IN_3O_4S$ 500.0141, found 500.0128. Anal. ($C_{18}H_{18}IN_3O_4S$) C, H, N.

Molecular Modeling. The enzymatic reaction of *T. gondii* adenosine kinase involves the bending motion of its two domains through induced fit movements. These domain movements are complex and apparently involve further tuning of the protein structure upon the binding of ATP. Therefore, the crystal structure of the enzyme−adenosine complex (1LII.pdb),[28] and not that of the apo-form, was used for our modeling calculation. In general, crystal structures lack hydrogen atoms, bond order, and sometimes few amino acid residues. We used the builder module of Maestro[43] to manually correct the structure of *T. gondii* adenosine kinase. This involved the adding of missing residues (239−240 and 255−269), hydrogen atoms, appropriate bond order, formal charges, and +2 for magnesium ion, as well as removing of water and fixing of proper atom-types (according to atomic hybridization). The manually inspected and corrected structure of *T. gondii* adenosine kinase was then subjected to a single run by Glide[44] protein preparation program to optimize the structure and ensure its chemical correctness. The determination of the binding mode of 7-deaza-6-benzylthioinosines was complicated by the fact that the enzyme functions via induced fit mechanism and the enzyme−-adenosine complex is not perfect for the studies because our analogues are almost ∼16 Å in length diagonally, whereas the diagonal length of the adenosine was ∼8 Å. Therefore, we used the induced fit docking[45] and the prime module of the Schrödinger Suite[46] to determine the binding mode of the 7-deaza-6-benzylthioinosines. After successful active-site modification and refinement by prime and redocking by Glide, enzyme−ligand complexes were subjected to energy minimization (EM)[47] to obtain the final binding mode.

All the calculations were performed on Schrödinger Suite 2006[46] (Schrödinger Inc.) with the GB/SA continuum water solvation model. OPLS_2001 and OPLS_2005 force fields were used for induced fit docking and then the same force fields were implemented in MACROMODEL 9.1 for energy minimization (EM). For EM studies, residues further than 16 Å from the adenosine binding site were frozen and residues from 6 to 16 Å were constrained by harmonic constraints. Only residues inside a 6 Å sphere from the nucleoside were allowed to move freely. EM calculations were performed for 5000 steps or until the energy difference between subsequent structures was 0.05 kJ/mol. The starting conformation for all of the ligands were obtained by 5000 steps of Monte Carlo conformational search under GB/SA continuum water solvation model and Polak−Ribiere conjugate gradient (PRCG) method used for the subsequent energy minimization.

Evaluation of the Newly Designed 7-Deaza-6-benzylthioinosine Analogues as Alternative Substrates for Purified *T. gondii* Adenosine Kinase. Enzyme assays were run under conditions where activity was linear with time and enzyme concentration.[22] Activity was determined by following the formation of radiolabeled AMP from adenosine. The assay mixture contains 50 mM Tris-Cl, pH 7.4; 2.5 mM ATP; 5 mM $MgCl_2$; 5 mM creatine phosphate; creatine kinase; 5 μM [8-^{14}C]adenosine (55 Ci/mol); 50 μL of purified *T. gondii* adenosine kinase; prepared as previously described[30] in a final volume of 100 μL, in the absence or presence of various concentrations of the compound under evaluation. Incubation was carried out at 37 °C and terminated by boiling in a water bath for 2 min, followed by freezing for at least 20 min. Precipitated proteins were removed by centrifugation and 10 μL of the supernatant was spotted on silica gel TLC plates. The TLC plates were developed in a mixture of chloroform/methanol/acetic acid (102:12:6 v/v/v). The R_f values were 0.27 for adenosine and 0.17 for AMP. The amounts of radioactivity in both the substrate and product were calculated on a percentage basis using computerized Berthold LB-284 automatic TLC-linear analyzers. Apparent K_i values of these analogues were calculated from Dixon plots $1/v$ vs [I] by least-squares fitting by computer programs written by Dr. Naguib as previously described.[21,22,24,25,27] The synthesis of the nucleoside 5′-monophosphates from the tested 7-deaza-6-benzylthioinosine analogues was confirmed by HPLC and NMR analyses as previously described,[22,25,27] indicating that these compounds are alternative substrates to *T. gondii* adenosine kinase. Since these compounds are alternative substrates of *T. gondii* adenosine kinase, their apparent K_i values are equal to their apparent K_m values[48] as presented in Table 1.

Evaluation of 7-Deaza-6-benzylthioinosine Analogues as Potential Antitoxoplasmosis Agents against Tachyzoites in Tissue Culture. The wild type RH and the adenosine kinase deficient mutant TgAK^{-3} [41] strains of *T. gondii* were used in these experiments. The adenosine kinase deficient mutant TgAK^{-3} was used as a control to verify that the promising drugs were metabolized by adenosine kinase in vivo as was the case in vitro. The effects of purine analogues as antitoxoplasmosis agents in tissue culture was measured by their ability to inhibit the replication of intracellular *T. gondii* in tissue culture, using monolayers of human foreskin fibroblasts (CRL-1634, American Type Culture Collection), grown for no more than 20 passages in RPMI 1640 medium.[23,25,27] The viability of intracellular parasites was evaluated by the selective incorporation of radiolabeled uracil into nucleic acids of the parasites at least in triplicate as previously described.[23,25,27] Briefly, confluent cells (4−5 days of incubation) were cultured for 24 h in the 24-well flat bottom microtiter plates ((∼5 × 10^5/1 mL)/well)) and incubated at 37 °C in 5% CO_2, 95% air to allow the cells to attach. The medium was then removed, and the cells were infected with isolated *T. gondii* in medium with 3% FBS (1 parasite/cell). After 1 h of incubation, the cultures were washed with media with 10% FBS to remove extracellular parasites. FBS was maintained at a final concentration of 10%. Compounds were dissolved in 50% ethanol and then added to cultures of the parasite-infected cells to give a final concentration of 0, 5, 10, 25, and 50 μM. The final concentration of ethanol when the compounds were added to the wells was 2.5%. After an additional 18 h incubation the medium was replaced with 1 mL of drug-free medium containing [5,6-^{13}H]uracil (5 μCi/mL) and incubated for another 6 h after which the medium was removed. The fibroblasts were then released from

the wells by trypsinization with the addition of 200 μL of trypsin/EDTA (2.5×) to each well. After 10 min of incubation, 1 mL of ice cold 10% trichloroacetic acid (TCA) was added to each well. The plates were then placed on a shaker to ensure the detachment of the cells. The suspended contents of each well were filtered through GF/A 2.4 cm glass microfiber filters (Whatman), which were prewashed each with 1 mL of doubly distilled H_2O and dried. After filtration, the filters were washed with 10 mL of methanol, left to dry, then placed in scintillation vials containing 5 mL of Econo-Safe scintillation fluor (Research Products International Corp, Mount Prospect, IL), and radioactivity was counted using an LS5801 Beckman scintillation counter. The effect of the compounds on the growth of the parasite was estimated as percentage reduction in the uptake of radiolabeled uracil by treated parasites compared to the untreated controls.[22,23,25,27,49] Radiolabel incorporation closely correlates with parasite growth.[22,23,25,27,49]

Host Toxicity of 7-Deaza-6-benzylthioinosine Analogues. Possible toxicity against the host cells by the same doses of the various analogues used in the above experiments was measured, at least in triplicate, using a modification of the microculture tetrazolium (MTT) assay on uninfected monolayers of human foreskin fibroblasts (grown for no more than 20 passages) in RPMI 1640 medium.[22,23,25,27] Briefly, confluent cells were incubated for at least 24 h in 96-well flat bottom microtiterplates (($\sim 10^5$/200 μL)/well)) at 37 °C in 5% CO_2, 95% air to allow the cells to attach. The medium was then replaced with 200 μL of fresh medium. The appropriate concentration of the compounds was dissolved in 50 μL of medium and added to each well to give the desired final concentrations. The cultures were then incubated for 48 h, after which 50 μL of sterile MTT solution (2 mg/1 mL PBS) was added to each well. MTT solution was sterilized by filtration through 0.22 μm filters (Costar, Cambridge, MA). After 4 h of incubation, the medium was removed and 100 μL of dimethyl sulfoxide (DMSO) was added to each well and the plates were shaken gently for 2−3 min to dissolve the formed formazan crystals. The absorbance was measured at 540 nm using a computerized microtiterplate reader (Themomax, Molecular Devices).

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Supporting Information Available: Elemental analysis data of compounds **6a**−**w**, **7**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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