Traceless Solid-Phase Synthesis and Biological Evaluation of Purine Analogs as Inhibitors of Multidrug Resistance Protein 4

Theresa May Chin Tan,*,† Fei Yang,† Han Fu,‡ Makam S. Raghavendra,‡ and Yulin Lam§,‡

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 117597, and Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543

Received June 21, 2006

The traceless solid-phase syntheses of 6-oxopurines and pyrazolo[3,4-*d*]pyrimidines are presented. The effects of these compounds on multidrug resistance protein 4 (MRP4/ABCC4) facilitated efflux was examined. Four of the compounds, **7b**, **7c**, **15a**, and **17e**, were active in inhibiting MRP4-mediated efflux of the bimane−glutathione conjugate. In addition, all four compounds were also able to reverse MRP4-mediated resistance to the anticancer drug 6-thioguanine. In the presence of 25 μM **15a** or **17e**, there was complete reversal. The reversal of resistance was achieved without any effects on the uptake and metabolism of 6-thioguanine.

Introduction

The ATP-binding cassette (ABC) transporters are membrane proteins that facilitate the transport of a diverse variety of molecules. In humans, there are 48 ABC transporters belonging to six different subfamilies.[1] Members of the human ABC superfamily have been implicated in multidrug resistance in chemotherapy. To date, P-glycoprotein (Pgp/MDR1/ABCB1) is the best studied in terms of its role in multidrug resistance.[2] Other human ABC transporters that are implicated in drug resistance include the breast cancer resistance protein (BCRP/ABCG2) and the multidrug resistance protein 1 (MRP1/ABCC1).[3,4]

The ABCC subfamily has 12 members, of which 9 are MRP proteins (MRP1-6/ABCC1-6 and MRP7-9/ABCC10-12).[5] Although most ABC proteins contain two transmembrane domains and two nucleotide binding domains, some MRP proteins also contain a third transmembrane domain at the N-terminal.[5] These include MRP1-3, MRP6 and MRP7. In contrast, MRP4, MRP5, MRP8, and MRP9 all have the typical two transmembrane domains and two nucleotide binding domains topology.

MRP1 was cloned in 1992, and studies with cell lines overexpressing MRP1 showed that MRP1 is able of conferring resistance to anthracyclines, vinca alkaloids, epipodophyllotoxins, camptothecins, and methotrexate.[6] However, to date, the expression of MRP1 in clinical samples has not been systematically examined, and the contribution of MRP1 to resistance to the anticancer drugs in humans is still not well-established.[3] Similar to the observations for MRP1, overexpression of MRP4 and MRP5, too, can confer resistance to therapeutic agents. However, unlike MRP1, MRP4 and MRP5 confer resistance to nucleoside and nucleobase analogs that are used therapeutically as anticancer or antiviral agents. These include compounds such as 6-mercaptopurine, 6-thioguanine (6TG), 9-(2-phosphonylmethoxyethyl)adenine (PMEA), and azidothymidine.[7] Overexpression of MRP4 also results in resistance to camptothecins.[8] In addition to therapeutic agents, MRP4 is also able to transport various endogenous molecules, including cAMP and cGMP, as well as conjugated steroids, bile acids, prostaglandins and glutathione.[7,9−12]

To date, various compounds have been shown to inhibit the activities of MRP4. These include probenecid, sulfinpyrazone, indomethacin, dipyridamole, and compounds containing the purine (e.g. 6TG and PMEA) and pyrazolo[4,3-*d*]pyrimidin-7-one templates (e.g. sildenafil).[12−15] These earlier works prompted us to synthesize other purine analogs, such as 6-oxopurines and pyrazolo[3,4-*d*]pyrimidines, and examine their inhibitory activities on human MRP4.

Methodologies for the preparation of purines and pyrazolo[3,4-*d*]pyrimidines have attracted much attention from both industry and academia, and various solution-phase syntheses of these compounds have been reported.[16] In recent years, linkage strategies for the preparation of purines on solid phase have also been examined; however these reported synthetic procedures have focused on the preparation of adenine analogs,[16a,17] and to our knowledge, no examples on the solid-phase synthesis of pyrazolo[3,4-*d*]pyrimidines have been reported. As part of a continuing effort toward the development of solid-phase synthesis (SPS) protocols for generation of purine libraries, we herein describe a traceless solid-phase procedure using the Wang resin which is applicable for the synthesis of both 6-oxopurines and pyrazolo[3,4-*d*]pyrimidines (Scheme 1).

Wang resin is an acid-labile *p*-alkoxybenzyl alcohol resin that is commonly used to prepare carboxylic compounds via

* To whom correspondence should be addressed. Fax: (65)-6779-1453. E-mail: bchtant@nus.edu.sg.

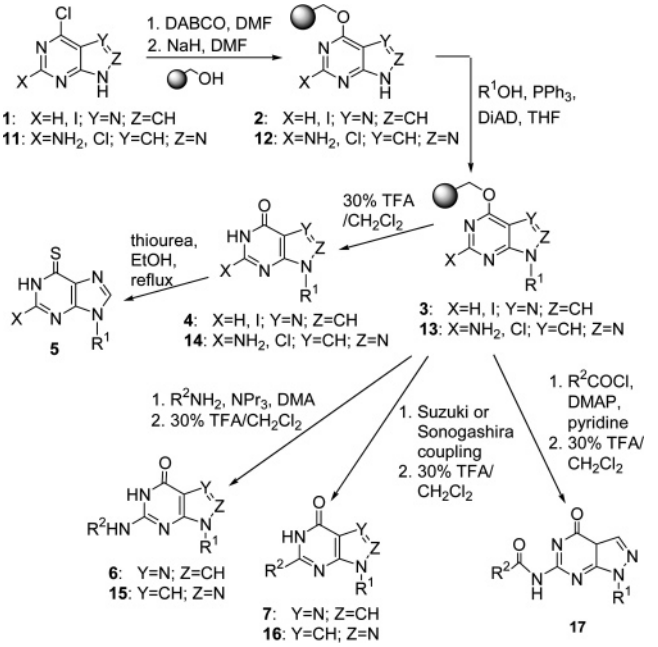
† Yong Loo Lin School of Medicine.

‡ Department of Chemistry.

§ This author was responsible for the nonbiological aspects of the research.

10.1021/cc060084t CCC: $37.00
Published on Web 12/29/2006

Scheme 1. SPOS of 6-Oxopurines and Pyrazolo[3,4-*d*]pyrimidines



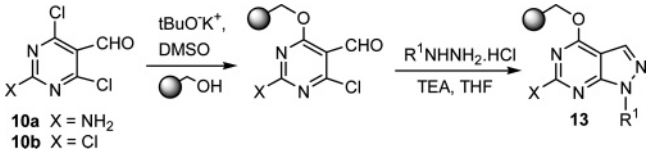
an ester linkage. Its application in the preparation of alcohols, phenols or hydroxamic acids via a benzyl ether linkage has also been reported.[18] Since the benzyl ether linkage is also stable to various reaction conditions, including the Grignard reactions, ester saponification with LiOH, Weinreb amide formation reactions, Mitsunobu reactions, and the Heck reaction,[18,19] we reasoned that the Wang resin would be an ideal solid support for our SPS strategy. We would expect the benzyl ether linker connected to the purine or pyrazolo-[3,4-*d*]pyrimidine framework to leave a hydroxyl group after acidic cleavage to give 6-oxopurines and pyrazolo[3,4-*d*]-pyrimidin-4-ones, respectively.

Results and Discussion

Solid-Phase Synthesis. Resins **2** and **12** were obtained by capturing 6-chloropurine **1** and 4-chloro-1*H*-pyrazolo-[3,4-*d*]pyrimidine **11**, respectively, on the Wang resin. Earlier studies have shown that the chloride can be easily replaced by benzyl thiol.[20] Since sulfur is a stronger nucleophile than oxygen, we needed to change the chloride to a better leaving group. A standard method involves the conversion of the chloride into an ammonio species by treatment with trimethylamine. However, the latter reagent is very volatile and toxic and, thus, is difficult to handle, especially on a large scale. Hence, 1,4-diazabicyclo-[2.2.2]octane (DABCO) was used instead.[21] Treatment of **1** and **11** with DABCO gave the DABCO−purine salt, which was subsequently reacted with the Wang resin in DMF at room temperature to give resins **2** and **12**, respectively.

Next, we examined the N-alkylation procedure. Attempts to N9-alkylate resin **2** with 1-bromobutane in the presence of NaH provided the N7 and N9 alkylated regioisomers in a 1:1 ratio (as shown in compounds **4c** and **6d**). To selectively alkylate the N9-position, resin **2** was treated with *n*-butanol under the Mitsunobu alkylation conditions. The formation of resin **3** was monitored by high-resolution magic-angle

Scheme 2. Synthesis of Resin **13**



spinning (HRMAS) gel-phase ^{13}C and DEPT135 NMR experiments for the appearance of peaks corresponding to the alkyl group. Corresponding application of the Mitsunobu reaction to resin **12** gave resin **13**, which could alternatively be prepared by capturing 2-amino-4,6-dichloropyrimidine-5-carbaldehyde **10a**[22] and 2,4,6-trichloropyrimidine-5-carbaldehyde **10b**[23] onto the Wang resin, followed by treatment with various substituted hydrazines in THF (Scheme 2). C2-amino groups were then introduced through the reaction of resins **3** (X = I) and **13** (X = Cl) with a variety of primary and secondary amines in DMA at 80 °C while C2-alkylation was achieved via Suzuki or Sonogashira coupling. It was interesting to note that unlike resin **3**, which was amenable to both Suzuki and Sonogashira coupling, resin **13** could be C2-alkylated only via the Suzuki coupling. (During the Sonogashira coupling, a black precipitate formed within 2 h of reaction. This precipitate could not be separated from the resin and may have affected the reaction.) The compounds were subsequently efficiently cleaved from the resin with 30% TFA in CH_2Cl_2 to give the target products. In the same vein, treatment of **13** (X = NH_2) with acid chloride in pyridine gave an *N*-(1,4-disubstituted) (3a,4-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)amide, which upon debenzylative cleavage provided **17**. In contrast, the solution-phase methodology gave predominantly the *N*,*N*-diamide, as demonstrated by **17e** and **17f**. To illustrate the versatility of this protocol, a set of 12 6-oxopurines (**4a**−**c**, **5a**, **5b**, **6a**−**d**, and **7a**−**c**) and 15 pyrazolo[3,4-*d*]pyrimidines (**14a**−**c**, **16a**, **16b**, **15a**−**f**, and **17a-d**) were prepared (Figure 1). Compounds **8** and **9** (Figure 1) were prepared according to reported procedures.[24,25]

Effects of 6-Oxopurines and Pyrazolo[3,4-*d*]pyrimidines on Bimane−GS Efflux. MRP4 is able to mediate the transport of conjugated molecules, including molecules conjugated with glucuronate or with glutathione.[12−14] We have previously shown that cells overexpressing MRP4 are able to mediate the efflux of the fluorescent bimane−glutathione (bimane−GS) adduct.[12] Thus, in this study, we first examined the effects of 17 6-oxopurine derivatives (**4a**−**c**, **5a**, **5b**, **6a**−**6e**, **7a**−**7c**, **8a**, **8b**, **9a**, and **9b**) on MRP4-mediated bimane−GS efflux. This was carried out using HepG2 cells stably overexpressing MRP4 (denoted as MRP4/HepG2). Cells stably transfected with the empty vector (denoted as V/HepG2) served as the control. As observed previously, MRP4/HepG2 cells were able to facilitate the efflux of bimane−GS at a significantly higher rate than could V/HepG2.[12]

Of the 17 6-oxopurines screened, only **7b** and **7c** were able to influence the bimane−GS transport ability of MRP4 at 100 μM. The presence of 25 μM **7b** led to significant reduction in MRP4-mediated transport (Table 1). The efflux was reduced from 7.7 to 2.9 nmol/mg protein. A significant inhibition of MRP4-mediated transport was also observed

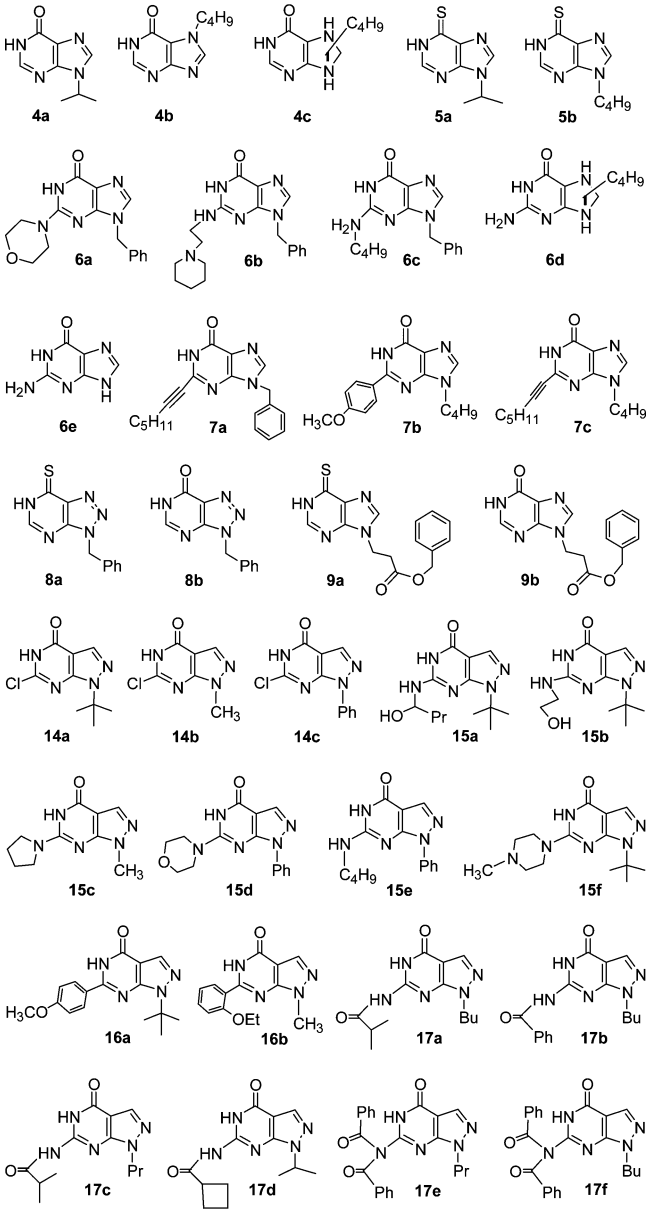


Figure 1. Library of 6-oxopurines and pyrazolo[3,4-*d*]pyrimidines.

in the presence of 25 μM **7c**, although the degree of inhibition was much less than that observed for **7b**. At 25 μM, neither **7b** nor **7c** affected efflux from the control V/HepG2 cells. In addition, the effects of both compounds were specific to the efflux process because there were no differences in the total bimane−GS synthesis between cells that were exposed to **7b** or **7c** and those that were not. A significant inhibition of MRP4-mediated transport was also observed at 50 μM, but at this concentration, there was also a significant reduction in the efflux from the control V/HepG2 cells.

The pyrazolo[3,4-*d*]pyrimidines were also examined to see if they could influence the MRP4-mediated transport of bimane−GS. Of the 17 pyrazolo[3,4-*d*]pyrimidines tested, 2 were found to affect the efflux of bimane−GS (Table 1). MRP4-faciliated efflux of bimane−GS was reduced from 7.5 nmol/mg protein to 4.5nmol/mg protein in the presence of 100 μM **15a**. At this concentration, there was no effect on the efflux from the V/HepG2 cells. There was also no effect on the total bimane−GS synthesis. In contrast, 150 μM **15a** led to significantly reduced efflux from the control V/HepG2 cells. The second pyrazolo[3,4-*d*]pyrimidine that was effective was compound **17e**. Bimane−GS efflux from MRP4/HepG2 was reduced from 7.7 to 4.4 nmol/mg protein in the presence of 150 μM **17e**. At concentrations up to 150 μM, compound **17e** had no effect on total bimane−GS synthesis. It also did not affect the efflux of bimane−GS from control V/HepG2 cells.

Cyclic nucleotides (cAMP, cGMP) and purine analogs, such as zaprinast and sildenafil at micromolar concentrations have been shown to inhibit MRP4-mediated transport of the glucuronide conjugate estradiol 17β-glucuronide.[13−14] In this study, the compounds **7b**, **7c**, **15a**, and **17e** were able to similarly inhibit the efflux of the glutathione conjugate bimane−GS. This inhibition was specifically on the efflux because the synthesis of bimane−GS was not affected by the compounds. A comparison of the effectiveness of these inhibitors revealed that **7b** was the best inhibitor of bimane−GS efflux and the inhibitory effect of **15a** and **17e** was comparable to 6TG (Table 2).

Effects of 6-Oxopurines and Pyrazolo[3,4-*d*]pyrimidines on 6TG Resistance. Given that MRP4 has the ability to mediate resistance to nucleoside and nucleobase analogs[14−15,26−27] and also to transport the phosphorylated metabolites of mercaptopurines,[26,28] it is highly possible that the expression of MRP4 may play a role in the pharmacokinetics of resistance to these therapeutic molecules. Thus, one of the aims of this study was to examine if it is possible to reverse the MRP4-mediated resistance to 6TG.

MRP4/HepG2 cells and V/HepG2 cells were first treated with 0−100 μM **7b**, **7c**, **15a**, or **17e** for 48 h to examine the effects on cell proliferation. As shown in Table 3, the IC_{50} for each of these compounds was >100 μM. There was no effect on cell growth and proliferation when the cells were exposed to 10 μM **7b** or the other three compounds at 25 μM for 48 h. Thus, these concentrations were used for the next series of experiments. The IC_{50} values for 6TG for the MRP4/HepG2 cells were significantly reduced in the presence of all four compounds (Table 4). In addition, compounds **15a** and **17e** were able to completely reverse the resistance conferred by MRP4. The concentrations used to achieve this reversal of resistance ranged from 10 to 25 μM. At these concentrations, the compounds had no adverse effects on cell proliferation and viability. The inhibitor concentrations at which the reversal of resistance was observed are similar to that which was previously reported for the inhibition of MRP4-mediated transport of another nucleoside analog, PMEA by sildenafil (IC_{50} of 20 μM).[13] It is also obvious that the purine analog inhibitors did not affect the uptake or metabolism of 6TG because the IC_{50} values in the control V/HepG2 cells were not affected by the presence of these analogs. In addition, at 25 μM, both **15a** and **17e** were able to selectively reverse resistance to 6TG without affecting MRP4-mediated efflux of bimane−GS. It is, indeed, promising to observe that it is possible to reverse resistance to 6TG without affecting the ability of MRP4 to transport conjugated molecules. Future studies are presently ongoing to understand the inhibition mechanism.

In summary, we have demonstrated a protocol for the traceless solid-phase synthesis of 6-oxopurines and pyrazolo-

Table 1. Effects of **7b**, **7c**, **15a**, and **17e** on Bimane−GS Efflux

concn (M)	total bimane−GS synthesized in V/HepG2 and MRP4/HepG2 (in parentheses) (nmol/mg protein)	bimane efflux from V/HepG2 cells (nmol/mg protein)	bimane efflux from MRP4/HepG2 cells (nmol/mg protein)	efflux mediated by MRP4[a] (nmol/mg protein)
		Compound **7b**		
0 (control)	48.2 ± 1.1 (48.5 ± 1.7)	14.6 ± 0.7	22.3 ± 1.0	7.7 ± 0.5
25	50.2 ± 3.5 (48.3 ± 3.4)	14.3 ± 1.0	17.1 ± 0.6[b]	2.9 ± 0.8[b]
50	50.0 ± 3.7 (49.5 ± 2.5)	11.8 ± 1.3[b]	16.0 ± 1.4[b]	4.3 ± 1.3[b]
		Compound **7c**		
0 (control)	50.5 ± 2.4 (49.2 ± 2.8)	15.6 ± 0.6	23.3 ± 0.9	7.7 ± 0.4
25	49.7 ± 2.4 (50.0 ± 3.8)	14.3 ± 0.8	19.7 ± 0.9[b]	5.4 ± 0.9[b]
50	51.0 ± 2.0 (50.2 ± 1.8)	13.1 ± 1.1[b]	19.2 ± 0.6[b]	6.1 ± 0.7[b]
		Compound **15a**		
0 (control)	57.1 ± 3.4 (56.8 ± 1.2)	16.2 ± 1.3	23.7 ± 0.5	7.5 ± 1.5
25	57.6 ± 1.7 (55.3 ± 1.0)	17.5 ± 0.9	22.9 ± 0.3	5.4 ± 0.8
50	54.2 ± 0.4 (53.5 ± 2.5)	16.8 ± 0.3	22.0 ± 1.1	5.2 ± 0.8[b]
100	57.6 ± 1.4 (58.9 ± 2.1)	16.1 ± 0.7	20.6 ± 0.3[b]	4.5 ± 0.6[b]
150	56.7 ± 1.4 (58.1 ± 2.2)	13.5 ± 1.8[b]	18.3 ± 1.5[b]	4.8 ± 0.3[b]
		Compound **17e**		
0 (control)	56.0 ± 2.9 (57.4 ± 2.5)	14.8 ± 0.8	22.5 ± 0.2	7.7 ± 0.9
25	57.7 ± 3.5 (59.5 ± 1.3)	15.4 ± 1.0	22.0 ± 0.4	6.6 ± 0.9
50	56.6 ± 1.1 (57.5 ± 2.1)	15.3 ± 0.4	21.5 ± 0.9	6.2 ± 1.2
100	57.1 ± 4.7 (60.7 ± 1.9)	15.2 ± 1.8.	21.3 ± 0.9	6.1 ± 1.3
150	59.2 ± 4.6 (58.4 ± 1.1)	13.8 ± 1.5	18.2 ± 0.9[b]	4.4 ± 1.2[b]

[a] The difference between the bimane efflux from V/HepG2 and those from MRP4/HepG2 cells represents the efflux mediate by MRP4. Data are expressed as mean ± SD from three independent experiments. [b] As compared with the corresponding untreated cells, $p < 0.05$, ANOVA analysis.

Table 2. Effects of Inhibitors on MRP4-Mediated Efflux of Bimane−GS

inhibitor	% of control
none	100[a]
100 μM 6TG	56 ± 9[b,c]
25 μM **7b**	37 ± 9[c]
25 μM **7c**	70 ± 14[c]
100 μM **15a**	60 ± 14[c]
150 μM **17e**	58 ± 18[c]

[a] Export in the absence of inhibitor was designated as 100%. [b] From ref 12. [c] As compared with export in the absent of an inhibitor, $p < 0.05$, ANOVA analysis.

[3,4-*d*]pyrimidine-4-ones with two points of diversification. The strategy benefits from the key role played by the benzyloxy linkage because it is a robust point of attachment and is stable to the reaction conditions for the variations at the C2 and N9 positions. Four of the compounds prepared were active in inhibiting MRP4-mediated efflux of bimane−glutathione conjugate. They were also able to reverse MRP4-mediated resistance to the anticancer drug 6-thioguanine, and for compounds **15a** and **17e**, complete reversal of the resistance conferred by MRP4 was observed at 25 μM.

Table 3. Viability of MRP4/HepG2 Cells (M) and V/HepG2 Cells (V) Following Exposure to **7b**, **7c**, **15a**, or **17e**[a]

	concn, μM				
	0	10	25	50	100
7b M	100%	94 ± 1.0%	87 ± 0.5%	77 ± 0.3%	76 ± 0.5%
V	100%	93 ± 0.1%	90 ± 4.6%	78 ± 2.2%	68 ± 3.0%
7c M	100%	nd	96 ± 0.3%	86 ± 0.4%	84 ± 0.9%
V	100%	nd	97 ± 2.3%	83 ± 0.8%	77 ± 2.3%
15a M	100%	nd	98 ± 6.1%	92 ± 6.8%	88 ± 4.2%
V	100%	nd	96 ± 3.3%	93 ± 3.3%	88 ± 2.6%
17e M	100%	nd	98 ± 1.5%	96 ± 1.7%	83 ± 5.1%
V	100%	nd	92 ± 2.1%	84 ± 2.7%	80 ± 2.6%

[a] The cells were exposed to compound **7b**, **7c**, **15a**, or **17e** at the concentrations indicated for 48 h. A 20-μL portion of MTS reagent was then added, and the cells were incubated at 37 °C for 1 h. The absorbance at 490 nm was measured. Each concentration was carried out in triplicate. Data are expressed as mean ± SD from three independent experiments.

Experimental Section

General Procedures. The Wang resin was purchased from Tianjin Nankai Hecheng Science and Technology Co (100−200 mesh, Catalog no. HCW02-1). All chemicals were

Table 4. IC_{50} for 6TG in the Presence of the Purine Derivatives[a]

	IC_{50} (μM)		
concn	MRP4/HepG2 (A)	V/HepG2 (B)	fold resistance (A/B)
10 μM **7b**	18.5 ± 0.3[b]	13.0 ± 1.1	1.4
25 μM **7c**	29.4 ± 0.9[b]	15.3 ± 1.0	1.9
25 μM **15a**	14.9 ± 0.6[b]	12.3 ± 1.4	1.2
25 μM **17e**	12.2 ± 0.5[b]	10.7 ± 0.6	1.1
control	37.1 ± 3.8	13.9 ± 0.6	2.7

[a] The cells were exposed to 6TG together with compound **7b**, **7c**, **15a**, or **17e** at the concentrations indicated for 48 h. A 20-μL portion of MTS reagent was then added, and the cells were incubated at 37 °C for 1 h. The absorbance at 490 nm was measured. IC_{50} is the concentration at which cell growth is inhibited by 50%. At least five 6TG concentrations were used to determine the IC_{50}. Each concentration was carried out in triplicate. Data are expressed as mean ± SD from three independent experiments. [b] As compared with the corresponding untreated cells, $p < 0.05$, ANOVA analysis.

obtained from commercial suppliers and were used without purification. Analytical TLC was carried out on precoated plates (Merck silica gel 60, F254) and visualized with UV light. Flash column chromatography was performed with silica (Merck, 230−400 mesh). NMR spectra (^{1}H and ^{13}C) were recorded at 298 K on a Bruker DPX300 or AMX500 Fourier transform spectrometer. Chemical shifts are expressed in δ (parts per million) relative to the internal standard of tetramethylsilane. EI or ESI mass spectra were measured on a VG Micromass 7035 spectrometer, and the HRMS data were obtained on a Finnigan MAT 95XL-T.

General Procedure for the Preparation of Resin-Bound Purines 2 and 12. Purine **1** or **11** (0.395 mmol) and DABCO (0.861 g, 7.673 mmol) were dissolved in DMF (10 mL). The reaction mixture was stirred rapidly at room temperature for 4 h. A suspension of the Wang resin (1.15 mmol/g, 0.6 g) in DMF was treated with sodium hydride (5 equiv) at room temperature for 4 h. The DABCO−purine mixture was then added, and the suspension was shaken at room temperature for 48 h. The resin was filtered; washed successively with DMF, H_2O, EtOH, CH_2Cl_2, and Et_2O; and dried in vacuo for 24 h.

General Procedure for the Alkylation of Resin-Bound Purines 3 and 13. Using NaH/RBr. Resin **2** (1.15 mmol) was swollen in DMF (15 mL) at room temperature for 30 min. NaH (1.2 equiv) was added, and the mixture was shaken for 1 h. Bromobutane (1.2 equiv) was added, and the reaction mixture was stirred slowly at 80 °C. After 6 h, the resin was filtered; washed successively with THF, EtOH, CH_2Cl_2, and Et_2O; and dried in vacuo for 24 h.

Via Mitsunobu Alkylation. Resin **2** or **12** (1.15 mmol) was swollen in THF (15 mL) at room temperature for 30 min. Alcohol (1.7 equiv) and PPh_3 (1.8 equiv) were added, and then DiAD (1.5 equiv) was added dropwise at ice-water bath temperature. The mixture was shaken at room temperature overnight. The resin was then filtered; washed successively with THF, EtOH, CH_2Cl_2, and Et_2O; and dried in vacuo for 24 h. HRMAS gel-phase ^{13}C NMR ($CDCl_3$) of **3** (X = I) showed the appearance of peaks corresponding to the butyl group: δ 13.55, 19.75, 31.81, 43.87. HRMAS gel-phase DEPT135 of **3** (X = H) showed the appearance of peaks corresponding to the butyl group: ^{13}C NMR (DMSO-d_6) δ 13.67, 19.87, 30.36, 47.46.

General Procedure for 2-Amination of Resin-Bound Purines 3 and 13. Resin **3** (X = I) or **13** (X = Cl) was swollen in DMA at room temperature for 30 min. The respective amine (3 equiv) and tripropylamine (3 equiv) were added, and the mixture was then stirred slowly at 80 °C under nitrogen for 24 h; filtered; washed successively with DMF, H_2O, EtOH, CH_2Cl_2, and Et_2O; and dried in vacuo for 24 h.

General Procedure for C2−C Bond Formation of Resin-Bound Purines 3 and 13. Via Sonogashira Coupling. Resin 3 (X = I) (0.12 g, 0.29 mmol) was swollen in DMA at room temperature for 30 min. To the suspension was added dichloro(1,2-bis(diphenylphosphino)ethane)-palladium(II) (1.1 equiv), DiEA (30 equiv), CuI (2.2 equiv), and the alkyne (20 equiv). The mixture was stirred slowly at 100 °C in the dark for 48 h and then filtered. The resin was washed successively with DMF, H_2O, EtOH, CH_2Cl_2, and Et_2O and dried in vacuo for 24 h.

Via Suzuki Coupling. Resin 3 (X = I) or 13 (X = Cl) (0.29 mmol) was swollen in DMF at room temperature for 30 min. To the suspension was added K_2CO_3 (2 equiv), Pd(PPh_3)$_4$ (0.1 equiv), and 4-methoxyphenylboronic acid (2 equiv). The reaction was stirred slowly at 100 °C in the dark for 48 h and then filtered. The resin was washed successively with DMF, H_2O, EtOH, CH_2Cl_2, and Et_2O and dried in vacuo for 24 h.

General Procedure for the Cleavage of Resin-Bound Purine. A suspension of resin in 30% TFA/CH_2Cl_2 was shaken at room temperature for 5 h. The resin was removed by filtration and washed with MeOH and CH_2Cl_2. The filtrate and washings were combined, neutralized with saturated aqueous $NaHCO_3$, concentrated, and purified by column chromatography (for 6-oxopurines, the eluent used was CH_3OH/CH_2Cl_2, 1:15−1:10; for pyrazolo[3,4-*d*]pyrimidines, the eluent used was ethyl acetate/hexane, 2:3).

General Procedure for the Sulfuration of 6-Oxopurines. Thiourea (8 equiv) was added to the solution of the 6-oxopurine in ethanol, and the reaction mixture was stirred under reflux conditions for 2 h, after which the mixture was cooled in an ice bath, and the solid that precipitated out was filtered and washed with ethanol.

Synthesis of 4-Chloro-6-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidine (11). To a solution of **10** (25 mmol) in THF/H_2O, 3:1(100 mL), at 50 °C was added hydrazine hydrate (75 mmol) in water (25 mL), and the mixture was stirred at room temperature for 10 min, after which the mixture was poured into ice−water and concentrated to half the volume. The precipitate obtained was filtered and dried under vacuum. **11** (X = NH_2): ^{1}H NMR (DMSO-d_6) δ 7.13 (s, 2H, NH_2), 7.94 (s, 1H, CH), 13.24 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 106.85, 133.70, 154.29, 158.50, 162.50. HRMS (EI): Calcd for $C_5H_4ClN_5$, 169.0155; found, 169.0157. Yield: 70%.

General Procedure for the Preparation of 1,6-Disubstituted 4-Benzyloxy-1*H*-pyrazolo[3,4-*d*]pyrimidine Resin (13) from Compound 10. Wang resin (2 g, 3.2 mmol) was swollen in DMSO (30 mL) for 30 min, after which potassium *tert*-butoxide (0.43 g, 4 mmol) was added, and the mixture

was shaken at room temperature for 1 h. The reaction mixture was cooled to 0−10 °C, a cooled solution of **10** (10 mmol) in DMSO (30 mL) was added, and the reaction mixture was shaken at room temperature for an additional 10 h. The resin was then filtered and successively washed with DMSO, H_2O, THF, and MeOH and dried in vacuo for 24 h.

The dried resin (1.6 mmol) was swollen in THF (20 mL) for 30 min and then treated with the respective substituted hydrazine (5 equiv) and triethylamine (15 equiv). The reaction mixture was shaken for 12 h, after which the resin was filtered and washed with THF and methanol and dried in vacuo for 24 h.

General Procedure for the N-Acylation of Resin 13. Resin **13** (X = NH_2, 0.8 mmol) was swollen in pyridine (20 mL) for 30 min, and DMAP (50 mg) was added. The mixture was cooled to 0 °C, and the respective acid chloride (4 mmol) was added dropwise over a period of 15 min, after which the mixture was shaken at room temperature for 6 h. The resin was filtered; washed with THF, water, and methanol; and dried in vacuo for 24 h.

9-Isopropyl-1,9-dihydropurin-6-one (4a). ^{1}H NMR (CD_3-OD) δ 1.61 (d, J = 6.6 Hz, 6H, CH_3 + CH_3), 8.03 (s, 1H, H-8), 8.15 (s, 1H, H-2). ^{13}C NMR (CD_3OD) δ 21.87 (×2), 48.67, 124.64, 139.05, 145.32, 148.95, 158.25. HRMS (EI) Calcd for $C_8H_{10}N_4O$, 178.0855; found, 178.0855. Overall yield: 47%.

***N*7-Butyl-6-oxopurine (4b).** ^{1}H NMR (CD_3OD) δ 0.99 (t, J = 7.3 Hz, 3H, CH_3), 1.31−1.44 (m, 2H, CH_2CH_3), 1.87−1.96 (m, 2H, CH_2CH_2N7), 4.45 (t, J = 7.1 Hz, 2H, CH_2N7), 8.06 (s, 1H, C8H), 8.22 (s, 1H, C2H). HRMS (EI) Calcd for $C_9H_{12}N_4O$, 192.1011; found, 192.1012. Overall yield: 22%.

Mixture of N7- and N9-Butyl-6-oxopurine (4c). ^{1}H NMR (CD_3OD) δ 0.94−0.99 (m, CH_3), 1.31−1.39 (m, CH2), 1.87−1.89 (m, CH_2), 4.25 (t, J = 7.1 Hz, N9CH_2), 4.42 (t, J = 7.2 Hz, N7CH_2), 7.99 (s, purine H), 8.03 (s, purine H), 8.06 (s, purine H), 8.17 (s, purine H). EI-MS m/z 243.2 (M^+); 192.1. Overall yield: 56%.

9-Isopropyl-1,9-dihydropurine-6-thione (5a). ^{1}H NMR (DMSO-d_6) δ 1.50 (d, 2H, J = 6.8 Hz, 2CH_3), 4.68−4.77 (m, 1H, CH), 8.19 (s, 1H, C8H), 8.39 (s, 1H, C2H), 13.70 (s, 1H, N1H). ^{13}C NMR ($CDCl_3$) δ 21.56 (×2), 46.67, 134.75, 140.56, 143.02, 144.04, 175.29. HRMS (EI) Calcd for $C_8H_{10}SN_4$, 194.0630; found, 194.0626. Yield for sulfuration: 90%.

9-Butyl-1,9-dihydropurine-6-thione (5b). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.2 Hz, CH_3), 1.19−1.26 (m, 2H, CH_2), 1.71−1.78 (m, 2H, CH_2), 4.15 (t, 2H, J = 7.2 Hz, N9CH_2), 8.19 (s, 1H, C8H), 8.30 (s, 1H, C2H), 13.69 (s, 1H, N1H) ^{13}C NMR ($CDCl_3$) δ 12.76, 18.64, 30.85, 42.56, 134.40, 142.53, 143.56, 144.26, 175.23. HRMS (EI) Calcd for $C_9H_{12}SN_4$, 208.0783; found, 208.0782. Yield for sulfuration: 92%.

9-Benzyl-2-morpholin-4-yl-1,9-dihydropurin-6-one (6a). ^{1}H NMR ($CDCl_3$) δ 3.78−3.79 (m + m, 4H + 4H, 2CH_2N + 2CH_2O), 5.19 (s, 2H, BnCH_2), 7.33−7.56 (m, 5H, ArH), 7.79 (s, 1H, CH). ^{13}C NMR ($CDCl_3$,) δ 45.55, 46.98, 66.34, 127.72, 128.28, 128.95, 132.97, 135.74, 137.98, 152.68, 159.18. HRMS (EI) Calcd for $C_{16}H_{17}N_5O_2$, 311.1382; found, 311.1380. Overall yield: 29%.

9-Benzyl-2-(2-piperidin-1-yl-ethylamino)-1,9-dihydropurin-6-one (6b). ^{1}H NMR (CD_3OD) δ 1.62 (m, 2H, H4′), 1.80−1.84 (m, 4H, H3′ + H5′), 3.30−3.32 (m, 2H, NHCH_2), 3.35−3.36 (m, 4H, $H_{2'}$ + $H_{6'}$), 3.69 (t, 2H, J = 5.8 Hz, N1′CH_2), 5.27 (s, 2H, BnCH_2), 7.29−7.34 (m, 5H, ArH), 7.81 (s, 1H, CH). ^{13}C NMR (CD_3OD) δ 23.37, 24.50(×2), 24.58, 37.47, 54.57(×2), 56.76, 117.41, 128.81, 129.17, 129.94, 137.76, 139.71, 152.52, 154.02, 159.42. HRMS (ESI, M + H) Calcd for $C_{19}H_{25}N_6O$, 353.2090; found, 353.2091. Overall yield: 26%.

9-Benzyl-2-butylamino-1,9-dihydropurin-6-one (6c). ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.3 Hz, CH_3), 1.28−1.38 (m, 2H, CH_2), 1.44−1.54 (m, 2H, CH_2), 3.24−3.32 (m, 2H, NHCH_2), 5.16 (s, 2H, BnCH_2), 7.33−7.34 (m, 5H, ArH), 7.79 (s, 1H, CH). ^{13}C NMR ($CDCl_3$) δ 13.16, 18.98, 30.38, 38.16, 45.52, 115.89, 127.17, 127.32, 128.05, 136.66, 136.73, 150.40, 152.27, 156.52. HRMS (EI) Calcd for $C_{16}H_{19}N_5O$, 297.1590; found, 297.1591. Overall yield: 32%.

Mixture of N7- and N9-Butylguanine (6d). ^{1}H NMR (CD_3OD) δ 1.02−1.07 (m, CH_3), 1.35−1.46 (m, CH_2), 1.49−1.95 (m, CH_2), 4.15 (t, J = 7.1 Hz, N9CH_2), 4.38 (t, J = 7.1 Hz, N7CH_2), 7.79 (s, C2H), 7.99 (s, C2H). EI-MS m/z 243.2 (M^+); 210.1. Overall yield: 53%.

9-Benzyl-2-hept-1-ynyl-1,9-dihydropurin-6-one (7a). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 7.1 Hz, CH_3), 1.25−1.66 (m, 6H, $(CH_2)_3$), 2.41 (t, 2H, J = 7.2 Hz, CCH_2), 5.59 (s, 2H, NCH_2), 7.36 (s, 5H, ArH), 8.10 (s, 1H, CH). ^{13}C NMR (CD_3OD) δ 13.87, 19.16, 22.09, 27.45, 31.03, 50.95, 74.19, 94.98, 128.12, 128.18, 128.74 (×2), 129.15 (×2), 135.27, 138.14, 154.81. HRMS (EI) Calcd for $C_{19}H_{20}N_4O$, 320.1637; found, 320.1633. Overall yield: 24%.

9-^{n}Butyl-2-(4-methoxyphenyl)-6-oxo-1,9-2*H*-purine (7b). ^{1}H NMR ($CDCl_3$) δ 0.98 (t, J = 7.3 Hz, 3H, CH_3), 1.33−1.46 (m, 2H, CH_2), 1.86−1.96 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.22 (t, J = 7.2 Hz, 2H, CH_2N9), 6.99 (d, J = 9.1 Hz, 2H, MeOAr*H*), 7.77 (s, 1H, H-8), 8.25 (d, J = 9.2 Hz, 2H, MeOAr*H*), 11.98 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 13.44, 19.76, 32.18, 43.63, 55.47, 114.46, 122.40, 124.55, 129.34, 139.89, 150.03, 153.13, 159.18, 162.39. HRMS (EI) Calcd for $C_{16}H_{18}N_4O_2$, 298.1430; found, 298.1432. Overall yield: 27%.

9-Butyl-2-hept-1-ynyl-1,9-dihydropurin-6-one (7c). ^{1}H NMR (CD_3OD) δ 0.87−0.98 (m, 6H, 2CH_3), 1.28−1.38 (m, 6H, 3CH_2), 1.60−1.71 (m, 2H, CH_2), 1.82−1.92 (m, 2H, CH_2), 2.49 (t, J = 7.1 Hz, 2H, CCH_2), 4.39 (t, J = 7.3 Hz, 2H, N9CH_2), 8.17 (s, 1H, C8H). ^{13}C NMR (CD_3OD) δ 13.84, 14.24, 19.72, 20.51, 23.22, 28.67, 30.74, 32.19, 34.41, 74.98, 95.41, 116.64, 130.69, 140.27, 145.67, 156.21. HRMS (EI) Calcd for $C_{16}H_{22}N_4O$, 286.1794; found, 286.1788. Overall yield: 28%.

3-Benzyl-3*H*-^{1,2,3}triazolo[4,5-*d*]pyrimidine-7(6*H*)-thione (8a). ^{1}H NMR (CD_3OD, 300 Hz) δ 5.77 (s, 2H, CH_2), 7.32−7.38 (m, 5H, Ph), 8.32 (s, 1H, C2H). ^{13}C NMR (DMSO-d_6, 300 Hz) δ (ppm): 49.87, 127.80, 128.16, 128.76, 135.13, 138.05, 144.00, 148.48, 177.63. HRMS (EI): Calcd for $C_{11}H_9N_5S$, 243.0579; found, 243.0575.

3-Benzyl-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (8b). ^{1}H NMR (DMSO-d_6, 300 Hz) δ 5.75 (s, 2H, CH_2), 7.31−7.37 (m, 5H, Ph), 8.26 (s, 1H, C2H), 12.71 (br, 1H, N1H). ^{13}C NMR (DMSO-d_6, 300 Hz) δ (ppm): 49.93, 127.94, 128.32, 128.94, 129.05, 135.46, 148.67, 150.08, 155.52. HRMS (EI): Calcd for $C_{11}H_9N_5S$, 243.0579; found, 243.0575.

Benzyl 3-(6-Thioxo-1,6-dihydropurin-9-yl)propanoate (9a). ^{1}H NMR (DMSO-d_6, 300 Hz) δ 3.04 (t, 2H, J = 7.0 Hz, N9CH2C*H*2), 4.43 (t, J = 7.0 Hz, 2H N9CH2), 5.06 (s, 2H, $PhCH_2$), 7.25−7.35 (m, 5H, ArH), 8.16 (s, 1H, C8H), 8.25 (s, 1H, C2H). ^{13}C NMR (DMSO-d_6, 300 Hz) δ (ppm): 33.04, 39.36, 65.37, 127.46, 127.57, 127.88, 134.45, 135.25, 142.57, 143.58, 144.36, 169.81, 175.27. HRMS (EI): Calcd for $C_{15}H_{14}N_4O_2S$, 314.0837; found, 314.0836.

Benzyl 3-(6-Chloro-9*H*-purin-9-yl)propanoate (9b). ^{1}H NMR (DMSO-d_6): δ 3.02 (t, J = 6.8 Hz, 2H, N9CH_2C*H*$_2$), 4.40 (t, J = 6.8 Hz, 2H, N9CH_2), 5.07 (s, 2H, $PhCH_2$), 7.26−7.35 (m, 5H, ArH), 8.02 (s, 1H, C8H), 8.04 (s, 1H, C2H), 12.26 (s, 1H, N1H). ^{13}C NMR ($CDCl_3$): δ (ppm) 33.27, 38.82, 65.34, 123.44, 127.46, 127.57, 127.89, 135.30, 139.81, 145.03, 147.82, 156.14, 169.86. HRMS (EI): Calcd for $C_{15}H_{14}N_4O_3$, 298.1066; found, 298.1079.

1-*tert*-Butyl-6-chloro-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (14a). ^{1}H NMR ($CDCl_3$) δ 1.74 (s, 9H, $3CH_3$), 8.05 (s, 1H, CH), 12.62 (s, NH, 1H). ^{13}C NMR ($CDCl_3$) δ 29.25, 61.40, 105.52, 133.40, 143.92, 151.21, 160.13. HRMS (EI): Calcd for $C_9H_{11}ClN_4O$, 226.0621; found, 226.0624. Yield: 58%.

6-Chloro-1-methyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (14b). ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 8.02 (s, 1H, CH), 13.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 35.20, 105.33, 135.41, 147.55, 152.17, 158.46. HRMS (EI): Calcd for $C_6H_5ClN_4O$, 184.0152; found, 184.0155. Yield: 50%.

6-Chloro-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (14c). ^{1}H NMR (DMSO-d_6) δ 7.42 (t, J = 7.3 Hz, 1H, ArH), 7.57 (t, J = 7.8 Hz, 2H, ArH), 7.93 (d, J = 7.7 Hz, 2H, ArH), 13.43 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 107.29, 123.01, 128.53, 130.35, 137.22, 138.81, 148.59, 152.08, 158.54. HRMS (EI): Calcd for $C_{11}H_7ClN_4O$, 246.0308; found, 246.0310. Yield: 54%.

1-*tert*-Butyl-6-(1-hydroxybutylamino)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (15a). ^{1}H NMR (DMSO-d_6) δ 0.90 (t, J = 7.4 Hz, 3H, CH_3), 1.41−1.61 (m, 2H, CH_2), 1.62 (s, 9H, $3CH_3$), 3.49−3.53 (m, 2H, CH_2), 3.74−3.77 (m, 1H, OH), 4.84−4.87 (m, 1H, CH), 6.40 (d, J = 8.0 Hz, 1H, NH), 7.66 (s, 1H, CH), 10.25 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.42, 24.77, 29.68, 54.82, 59.88, 62.64, 101.97, 133.36, 153.07, 154.77, 158.88. HRMS (EI) Calcd for $C_{13}H_{21}N_5O_2$, 279.1695; found, 279.1696. Yield: 23%.

1-*tert*-Butyl-6-(2-hydroxyethylamino)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (15b). ^{1}H NMR (DMSO-d_6) δ 1.60 (s, 9H, $3CH_3$), 3.39−3.41 (m, 2H, NCH_2), 3.61−3.63 (m, 2H, OCH_2), 4.52 (bs, 1H, OH), 6.35 (bs, 1H, NH), 7.58 (s, 1H, CH), 10.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 33.69, 48.24, 64.01, 64.89, 102.03, 137.23, 154.65, 157.16, 163.65. HRMS (EI) Calcd for $C_{11}H_{17}N_5O_2$, 251.1382; found, 251.1382. Yield: 15%.

1-Methyl-6-pyrrolidin-1-yl-1,5-dihydropyrazolo[3,4-*d*]-pyrimidin-4-one (15c). ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 4H, $2CH_2$), 3.45−3.49 (m, 4H, $2NCH_2$), 3.69 (s, 3H, CH_3), 7.72 (s, 1H, CH), 10.52 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 25.79, 34.13, 38.56, 47.88, 99.76, 134.90, 152.75, 154.58, 155.32, 159.58. HRMS (EI) Calcd for $C_{10}H_{13}N_5O$, 219.1120; found, 219.1119. Yield: 40%.

6-Morpholin-4-yl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]-pyrimidin-4-one (15d). ^{1}H NMR (DMSO-d_6) δ 3.65−3.69 (m, 8H, $4CH_2$), 7.30 (t, J = 7.1 Hz, 1H, ArH), 7.51 (t, J = 7.7 Hz, 2H, ArH), 8.06 (s, 1H, CH), 8.09 (s, 2H, ArH), 11.11 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 46.35, 66.61, 102.03, 121.61, 127.01, 130.11, 137.07, 140.00, 154.85, 155.01, 159.46. HRMS (EI) Calcd for $C_{15}H_{15}N_5O_2$, 297.1226; found, 297.1230. Yield: 44%.

6-Butylamino-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (15e). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.1 Hz, 3H, CH_3), 1.26−1.42 (m, 2H, CH_2), 1.52−1.61 (m, 2H, CH_2), 3.31−3.33 (m, 2H, NCH_2), 6.30 (s, 1H, NH), 7.22−7.27 (m, 1H, ArH), 7.39 (t, J = 7.8 Hz, 2H, ArH), 7.98 (s, 1H, CH), 8.05 (d, J = 8.0 Hz, 2H, ArH), 10.96 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 13.64, 19.98, 30.96, 41.08, 102.05, 121.01, 126.08, 128.71, 135.77, 138.95, 153.15, 155.06, 160.53. HRMS (EI) Calcd for $C_{15}H_{17}N_5O$, 283.1433; found, 283.1434. Yield: 35%.

1-*tert*-Butyl-6-(4-methylpiperazin-1-yl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (15f). ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 9H, $3CH_3$), 3.42 (s, 3H, CH_3), 3.91 (br s, 8H, $4CH_2$), 8.36 (s, 1H, CH), 11.81(s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 29.85, 43.25, 43.72, 52.63, 60.21, 102.43, 133.46, 152.89, 153.75, 159.39, 159.65, 159.81. HRMS (EI) Calcd for $C_{14}H_{22}N_6O$, 290.1855; found, 290.1853. Yield: 48%.

1-*tert*-Butyl-6-(4-methoxyphenyl)-1,5-dihydropyrazolo-[3,4-*d*]pyrimidin-4-one (16a). ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 9H, $3CH_3$), 3.89 (s, 3H, OCH_3), 7.05 (d, J = 8.7 Hz, 2H, ArH), 8.06 (s, 1H, ArH), 8.21 (d, J = 8.7 Hz, 2H, ArH), 11.62 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 28.06, 54.14, 102.09, 112.10, 113.01, 123.24, 127.97, 131.70, 151.20, 156.72, 159.01, 161.24. HRMS (EI) Calcd for $C_{16}H_{18}N_4O_2$, 298.1430; found, 298.1434. Yield: 18%.

6-(2-Ethoxyphenyl)-1-methyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (16b). ^{1}H NMR ($CDCl_3$) δ 1.60 (t, J = 7.0 Hz, 3H, CH_3), 4.04 (s, 3H, NCH_3), 4.31 (q, J = 10.5 Hz, 2H, CH_2), 7.05 (d, J = 8.4 Hz, 1H, ArH), 7.14 (t, J = 7.3 Hz, 1H, ArH), 7.50 (t, J = 8.7 Hz, 1H, ArH), 8.06 (s, 1H, CH), 8.51, 8.55 (dd, J = 8.0 Hz, J = 1.7 Hz, 1H, ArH), 11.11 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 14.62, 34.15, 65.40, 104.29, 113.01, 121.70, 128.20, 130.55, 131.31, 133.54, 134.72, 153.32, 157.32, 157.78. HRMS (EI) Calcd for $C_{14}H_{14}N_4O_2$, 270.1117; found, 270.1109. Yield: 17%.

***N*-(1-Butyl-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)-isobutyramide (17a).** ^{1}H NMR ($CDCl_3$) δ 0.80 (t, J = 7.3 Hz, 3H, CH_3), 1.12−1.17 (m, 2H, CH_2), 1.22 (d, J = 7.0 Hz, 6H, $2CH_3$), 1.67−1.77 (m, 2H, CH_2), 2.72−2.79 (m, 1H, CH), 4.08 (t, J = 7.1 Hz, 2H, NCH_2), 7.98 (s, 1H, CH), 9.85 (s, 1H, NH), 12.04 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 13.39, 18.92, 19.61, 31.37, 36.23, 46.92, 102.86,

135.01, 149.03, 151.71, 157.37, 179.78. HRMS (EI): Calcd for $C_{13}H_{19}N_5O_2$, 277.1539; found, 277.1538. Yield: 26%.

***N*-(1-Butyl-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)-benzamide (17b).** ^{1}H NMR ($CDCl_3$) δ 0.91 (t, $J = 7.3$ Hz, 3H, CH_3), 1.23−1.35 (m, 2H, CH_2), 1.78−1.88 (m, 2H, CH_2), 4.19 (t, $J = 7.1$ Hz, 2H, NCH_2), 7.55 (t, $J = 7.5$ Hz, 2H, ArH), 7.66 (t, $J = 7.3$ Hz, 1H, ArH), 7.95 (d, $J = 8.4$ Hz, 2H, ArH), 7.99 (s, 1H, CH), 9.03 (s, 1H, NH), 11.94 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 13.55, 19.78, 31.53, 47.10, 103.24, 127.56, 129.28, 131.43, 133.89, 135.21, 148.66, 151.29, 156.76, 167.75. HRMS (EI): Calcd for $C_{16}H_7N_5O_2$, 311.1382; found, 311.1383. Yield: 28%.

***N*-(4-Oxo-1-propyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)-isobutyramide (17c).** ^{1}H NMR ($CDCl_3$) δ (ppm): 0.74 (t, $J = 7.3$ Hz, 3H, CH_3), 1.23 (d, $J = 7.1$ Hz, 6H, $2CH_3$), 1.75−1.77 (m, 2H, CH_2), 2.73−2.80 (m, 1H, CH), 4.06 (t, $J = 7.3$ Hz, 2H, NCH_2), 7.99 (s, 1H, CH), 9.92 (s, 1H, NH), 12.03(s, NH, 1H). ^{13}C NMR ($CDCl_3$) δ 10.95, 18.95, 22.72, 36.19, 48.72, 102.86, 135.17, 148.99, 151.90, 157.42, 179.86. HRMS (EI): Calcd for $C_{12}H_{17}N_5O_2$, 263.1382; found, 263.1380. Yield: 24%.

Cyclobutanecarboxylic Acid (1-Isopropyl-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)-amide (17d). ^{1}H NMR ($CDCl_3$) δ 1.44 (d, $J = 6.6$ Hz, 6H, $2CH_3$), 1.92−2.10 (m, 2H, CH_2), 2.22−2.27 (m, 4H, $2CH_2$), 3.24−3.35 (m, 1H, $CH_{isopropyl}$), 4.69−4.78 (m, 1H, $CH_{cyclobutyl}$), 8.00 (s, 1H, CH), 8.67 (s, 1H, NH), 11.84 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 17.98, 21.84, 24.89, 40.36, 49.04, 103.14, 134.98, 148.40, 150.56, 157.05, 176.64. HRMS (EI): Calcd for $C_{13}H_{17}N_5O_2$, 275.1382; found, 275.1379. Yield: 32%.

General Solution-Phase Procedure for the N-Acylation of 4-Benzyloxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ylamine. To an ice-cold solution of 1-substituted 4-benzyloxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ylamine (1 mmol) and DMAP (0.050 g) in pyridine (20 mL) was slowly added benzoyl chloride (0.15 g, 2 mmol). The temperature of the mixture was gradually raised from 0 °C to room temperature within 2 h, after which the mixture was concentrated to dryness, and water was added to the residue. The solution was extracted repeatedly with ethyl acetate, and the combined ethyl acetate layer was dried over anhydrous $MgSO_4$, concentrated, and purified by column chromatography.

***N*-Benzoyl-*N*-(4-oxo-1-propyl-4,5-dihydro-1*H*-pyrazolo-[3,4-*d*]pyrimidin-6-yl)-benzamide (17e).** ^{1}H NMR (DMSO-d_6) δ 0.32 (t, $J = 7.3$ Hz, 3H, CH_3), 1.39−1.43 (m, 2H, CH_2), 3.97 (t, $J = 6.6$ Hz, 2H, NCH_2), 7.52 (t, $J = 7.5$ Hz, 4H, ArH), 7.62 (q, $J = 7.5$ Hz, 2H, ArH), 7.79 (d, $J = 8.4$ Hz, 4H, ArH), 8.04 (s, 1H, CH), 13.26 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.38, 23.23, 49.51, 104.90, 129.78, 130.02, 134.34, 134.46, 135.43, 149.87, 151.93, 158.73, 172.36. HRMS (EI): Calcd for $C_{22}H_{19}N_5O_3$, 401.1488; found, 401.1488. Yield for acylation: 78%. Overall yield: 23%.

***N*-Benzoyl-*N*-(1-butyl-4-oxo-4,5-dihydro-1*H*-pyrazolo-[3,4-*d*]pyrimidin-6-yl)-benzamide (17f).** ^{1}H NMR ($CDCl_3$) δ 0.68 (t, $J = 7.1$ Hz, 3H, CH_3), 0.79−0.88 (m, 2H, CH_2), 1.39−1.48 (m, 2H, CH_2), 3.99 (t, $J = 7.0$ Hz, 2H, CH_2), 7.41 (t, $J = 7.5$ Hz, 4H, ArH), 7.50 (q, $J = 7.4$ Hz, 3H, ArH), 7.86 (d, $J = 8.71$ Hz, 4H, ArH), 12.87 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 13.29, 19.27, 31.19, 47.28, 103.62, 128.81, 129.06, 133.23, 133.55, 134.47, 148.49, 151.29, 159.85, 171.46. HRMS (EI): Calcd for $C_{23}H_{25}N_5O_3$, 414.1644; found, 414.1644. Yield for acylation: 76%. Overall yield: 21%.

Cell Lines and Culture Conditions. HepG2 cells stably expressing the human MRP4 protein were previously described.[27] The MRP4 clone used in this study was MRP4/HepG2. The blasticidine clone, V/HepG2, which was transfected with the pcDNA6 vector, was included as the control. Cells were routinely grown in complete medium consisting of Dulbecco's modified Eagle's medium (DMEM), 1 mM sodium pyruvate, 2 mM glutamine, 0.1 mM nonessential amino acids, 100 units/mL of penicillin, 100 μg/mL of streptomycin, 10% fetal bovine serum, and 0.25 μg/mL of blasticidin. The cells were grown at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Bimane−Glutathione Efflux. The measurement of bimane−GS synthesis and efflux from MRP4/HepG2 and V/HepG2 cells was carried out as previously described.[12] In brief, cells were seeded in triplicate at a density of 6×10^5 cells per well into 6-well plates and incubated at 37 °C for 24 h. The cells were then incubated with 1 mL of DMEM medium containing 100 μM monochlorobimane (MCB) at 10 °C for 60 min with different concentrations of the purine derivatives. Controls consisted of cells incubated with DMSO, which was used to dissolve the compounds. After pretreatment with MCB, the plates were placed on ice, the medium was removed, and the cells were washed with cold Hanks' balanced salt solution (HBSS), without glucose twice. The cells were then incubated with 0.6 mL of HBSS containing 5.6 mM glucose and different concentrations of synthesized compounds at 37 °C for 5 min. A 0.2-mL portion of the incubation buffer and 0.2 mL of the cell lysate (in 0.2% sodium dodecyl sulfate, SDS) were collected. The bimane−GS content in the sample was measured by determining the fluorescence intensity at an excitation wavelength of 385 nm and an emission wavelength of 478 nm in a Gemini XS microplate spectrofluorometer from Molecular Devices Corp, U.S.A. A series of bimane−GS standards was used to generate a calibration curve for quantifying the amount of bimane−GS. The protein determination was carried out using the Bio-Rad Protein Dye with bovine serum albumin dissolved in 0.2% SDS as the standard.

Effects of the Purine Derivatives on 6TG Resistance. Cells were plated in triplicate at a density of 4×10^3 per well in a 96-well tissue culture plate. After 24 h of incubation at 37 °C, the cells were treated with 6TG, the purine derivative, or both. Control cells were incubated either with medium containing 0.1% DMSO or in medium only. Forty-eight hours later, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium/phenazine ethosulfate (MTS/PES) reagent (Promega) was added to each well. After incubation at 37 °C for 60 min, absorbance was measured at 490 nm. The data were used to calculate the 50% growth inhibitory concentration (IC_{50}). At least five 6TG concentrations were used to determine the IC_{50} value.

Acknowledgment. We thank the National University of Singapore for financial support of this work.

Supporting Information Available. Crystallographic files in CIF format of **4a**, **16a**, **17b**, and **17d** and all the NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dean, M.; Annilo, T. *Annu. Rev. Genomics Hum. Genet.* **2005**, *6*, 123−142.
(2) Gottesman, M.; Ling, V. *FEBS Lett.* **2006**, *580*, 998−1009.
(3) Polgar, O.; Bates, S. *Biochem. Soc. Trans.* **2005**, *33*, 241−245.
(4) Haimeur, A.; Conseil, G.; Deeley, R.; Cole, S. *Curr. Drug Metab.* **2004**, *5*, 21−53.
(5) Kruh, G.; Belinsky, M. *Oncogene* **2003**, *22*, 7537−7552.
(6) Deeley, R. Cole, S. *FEBS Lett.* **2006**, *580*, 1103−1111.
(7) Ritter, C.; Jedlitschky, G.; Meyer zu Schwabedissen, H.; Grube, M.; Kock, K.; Kroemer, H. *Drug Metab. Rev.* **2005**, *37*, 253−278.
(8) Tian, Q.; Zhang, J.; Tan, T.; Chan, E.; Duan, W.; Chan, S.; Boelsterli, U.; Ho, P.; Yang, H.; Bian, J.; Huang, M.; Zhu, Y.; Xiong, W.; Li, X.; Zhou, S. *Pharm. Res.* **2005**, *22*, 1837−1853.
(9) Zelcer, N.; Reid, G.; Wielinga, P.; Kuil, A.; van der Heijden, I.; Schuetz, J.; Borst, P. *Biochem. J.* **2003**, *371*, 361−367.
(10) Rius, M.; Hummel-Eisenbeiss, J.; Hofmann, A.; Keppler, D. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2006**, *290*, G64064−9.
(11) Reid, G.; Wielinga, P.; Zelcer, N.; van der Heijden, I.; Kuil, A.; de Haas, M.; Wijnholds, J.; Borst, P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9244−9249.
(12) Bai, J.; Lai, L.; Yeo, H.; Goh, B.; Tan, T. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 247−257.
(13) Reid, G.; Wielinga, P.; Zelcer, N.; De Haas, M.; van Deemter, L.; Wijnholds, J.; Balzarini, J.; Borst, P. *Mol. Pharmacol.* **2003**, *63*, 1094−1103.
(14) Chen, Z.; Lee, K.; Kruh, G. *J. Biol. Chem.* **2001**, *276*, 33747−33754.
(15) Adachi, M.; Sampath, J.; Lan, L.; Sun, D.; Hargrove, P.; Flatley, R.; Tatum, A.; Edwards, M.; Wezeman, M.; Matherly, L.; Drake, R.; Schuetz, J. *J. Biol. Chem.* **2002**, *277*, 38998−39004.
(16) (a) Bork, J. T.; Lee, J. W.; Chang, Y-T. *QSAR Comb. Sci.* **2004**, *23*, 245−260. (b) Dumaitre, B. A.; Dodie, N. Eur. Patent EP0636626A1, 1995. (c) Seela, F.; Steker, H. *Helv. Chim. Acta* **1986**, *69*, 1602−1613.
(17) (a) Nugiel, D. A.; Cornelius, L. A. M.; Corbett, J. W. *J. Org. Chem.* **1997**, *62*, 201−203. (b) Brill, W. K. D.; Toniolo, C. R. *Tetrahedron Lett.* **2001**, *42*, 6279−6282. (c) Lucrezia, R. D.; Gilbert, I. H.; Floyd, C. D. *J. Comb. Chem.* **2000**, *2*, 249−253. (d) Hammarstrom, L. G. J.; Meyer, M. E.; Smith, D. B.; Talamas, F. X. *Tetrahedron Lett.* **2003**, *44*, 8361−8363. (e) Ding, S.; Ding, Q.; Gray, N. S.; Schultz, P. G. U.S. Patent US20030171583, 2003.
(18) (a) Hanessian, S.; Ma, J.; Wang, W. *Tetrahedron Lett.* **1999**, *40*, 4631−4634. (b) Sarshar, S.; Siev, D.; Mjalli, A. M. M. *Tetrahedron Lett.* **1996**, *37*, 835−838. (c) Salives, R.; Dupas, G.; Ple, N.; Queguiner, G.; Turck, A.; George, P.; Sevrin, M.; Almario, A.; Li, A. *J. Comb. Chem.* **2005**, *7*, 414−420. (d) Rolland, C.; Hanquet, G.; Ducep, J. B.; Solladie, G. *Tetrahedron Lett.* **2001**, *42*, 7563−7566. (e) Schobert, R.; Jagusch, C. *Tetrahedron Lett.* **2003**, *44*, 6449−6451. (f) Raghavan, S.; Tony, K. A.; Reddy, S. R. *Tetrahedron Lett.* **2001**, *42*, 8383−8386. (g) Floyd, C. D.; Lewis, C. N.; Patel, S. R.; Whittaker, M. *Tetrahedron Lett.* **1996**, *37*, 8045−8048. (h) Stanger, K. J.; Krchnak, V. *J. Comb. Chem.* **2006**, *8*, 435−439. (i) Richter, L. S.; Desai, M. C. *Tetrahedron Lett.* **1997**, *38*, 321−322.
(19) Hanessian, S.; Xie, F. *Tetrahedron Lett.* **1998**, *39*, 737−740.
(20) Brun, V.; Legraverend, M.; Grierson, D. S. *Tetrahedron* **2002**, *58*, 7911−7923.
(21) Lembicz, N. K.; Grant, S.; Clegg, W.; Griffin, R. J.; Heath, S. L.; Golding, B. T. *J. Chem. Soc. Perkin Trans. 1* **1997**, 185−186.
(22) Seela, F.; Steker, H. *Helv. Chim. Acta* **1986**, *69*, 1602−1613.
(23) Yoneda, F.; Sakuma, Y.; Mizumoto, S.; Ito, R. *J. Chem. Soc. Perkin Trans. 1* **1976**, 1805−1808.
(24) Baindur, N.; Chadha, N.; Player, M. R. *J. Comb. Chem.* **2003**, *5*, 653−659.
(25) Fu, H.; Lam, Y. *J. Comb. Chem.* **2005**, *7*, 734−738.
(26) Schuetz, J.; Connelly, M.; Sun, D.; Paibir, S.; Flynn, P.; Srinivas, R.; Kumar, A.; Fridland, A. *Nat. Med*. **1999**, *5*, 1048−1051.
(27) Lai, L.; Tan, T. *Biochem. J.* **2002**, *361*, 497−503.
(28) Wielinga, P.; Reid, G.; Challa, E.; van der Heijden, I.; van Deemter, L.; de Haas, M.; Mol, C.; Kuil, A.; Groeneveld, E.; Schuetz, J.; Brouwer, C.; de Abreu, R.; Wijnholds, J.; Beijnen, J.; Borst, P. *Mol. Pharmacol.* **2002**, *62*, 1321−1331.

CC060084T