



The Synthesis and Biological Evaluation of 4-*p*-Nitrobenzylthio-*v*-triazolo[4,5-*d*]pyridazine and Imidazo[4,5-*d*]pyridazine Ribosides as Potential Nucleoside Transport Inhibitors†

Jacqueline C. Bussolari,^a Johanna D. Stoeckler^{b,‡} and Raymond P. Panzica^{a,*}

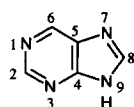
^aDepartments of Medicinal Chemistry and Chemistry, University of Rhode Island, Kingston, RI 02881, U.S.A.

^bDivision of Biology and Medicine, Brown University, Providence, RI 02912, U.S.A.

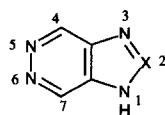
Abstract—The synthesis of *S*⁴-substituted nucleosides possessing the imidazo- and *v*-triazolo[4,5-*d*]pyridazine ring systems was undertaken and the compounds prepared were evaluated as inhibitors of nucleoside transport into human erythrocytes. 1-(2,3,5-Tri-*O*-acetyl-β-*D*-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione and 1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)imidazo[4,5-*d*]pyridazine-4(5*H*)-thione were each synthesized by two different routes and served as precursors for the title analogues. The nitrobenzylmercaptapurine riboside (NBMPR) analogues, 4-(*p*-nitrobenzylthio)-1-(β-*D*-ribofuranosyl)imidazo[4,5-*d*]pyridazine and 4-(*p*-nitrobenzylthio)-1-(β-*D*-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine, inhibited the transport of adenosine, but were approximately 4- and 28-fold less active, respectively, than NBMPR and nitrobenzylthioformycin, known potent and specific inhibitors of carrier-mediated transport. Copyright © 1996 Elsevier Science Ltd

Introduction

Ring modification of heterocycles has played a pivotal role in their development as effective chemotherapeutic agents. Our laboratory has focused on analogues of purines, especially those of the imidazo- and *v*-triazolo[4,5-*d*]pyridazine ring systems¹ (depicted below), as possible drug candidates/antimetabolites² and for their unique chemical properties.³ Recently, we have identified two imidazo[4,5-*d*]pyridazines (i.e. imidazo[4,5-*d*]pyridazin-4-one and imidazo[4,5-*d*]pyridazine-4-thione) as potent inhibitors of xanthine and guanine phosphoribosyltransferase, salvage enzymes isolated from *Toxoplasma gondii*.⁴



Purine



Imidazo[4,5-*d*]pyridazine X = CH
v-Triazolo[4,5-*d*]pyridazine X = N

In the present study, we concentrated on the design, preparation, and biological evaluation of selected *S*⁴-substituted imidazo- and *v*-triazolo[4,5-*d*]pyridazine nucleosides as inhibitors of nucleoside transport. The

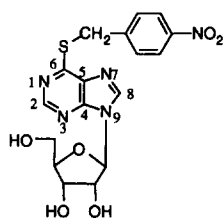
application of nucleoside transport inhibitors to modulate the activity of antimetabolites used for cancer treatment continues to attract attention.⁵ Two specific inhibitors of nucleoside transport are nitrobenzylmercaptapurine riboside⁶ (NBMPR, **1**) and nitrobenzylthioformycin⁷ (NBTF, **2**). A serious drawback associated with NBMPR is its likely bioconversion to 6-thioinosine. This thionucleoside is readily cleaved by purine nucleoside phosphorylase to 6-mercaptapurine. 6-Mercaptapurine has been shown to be immunosuppressive,⁸ as well as mutagenic⁹ and thus, long-term usage of NBMPR, even at non-toxic doses, remains a concern. On the other hand, bioconversion of NBTF would yield a thio-*C*-nucleoside that is resistant to phosphorylase cleavage. Yet, development of NBTF is hampered by the high cost and limited availability of formycin B, its natural product precursor. It is noteworthy that the synthetic methodology^{2b,10} leading to the title nucleosides provides them in sufficient quantity. Like NBTF their 6-thioinosine analogues (**6a** and **b**) are highly resistant to purine nucleoside phosphorylase. We now report the syntheses of 4-(*p*-nitrobenzylthio)-1-(β-*D*-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (**11a**) and 4-(*p*-nitrobenzylthio)-1-(β-*D*-ribofuranosyl)imidazo[4,5-*d*]pyridazine (**11b**) and their biological evaluation as inhibitors of human erythrocytic nucleoside transport. The syntheses of other related *S*⁴-substituted derivatives are also described.

Chemistry

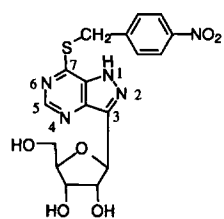
The synthetic pathways to the targeted nucleoside transport inhibitors rely on the inosine analogues 1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-*v*-triazolo[4,5-*d*]-

†Dedicated to the memory of Professor George U. Yuen and Professor William J. Burke.

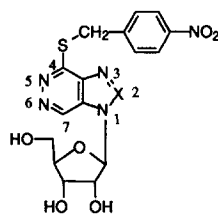
‡Present address: Roger Williams Medical Center, Experimental Pathology, 850 Chalkstone Avenue, Providence, RI 02908, U.S.A.



NBMPR, 1

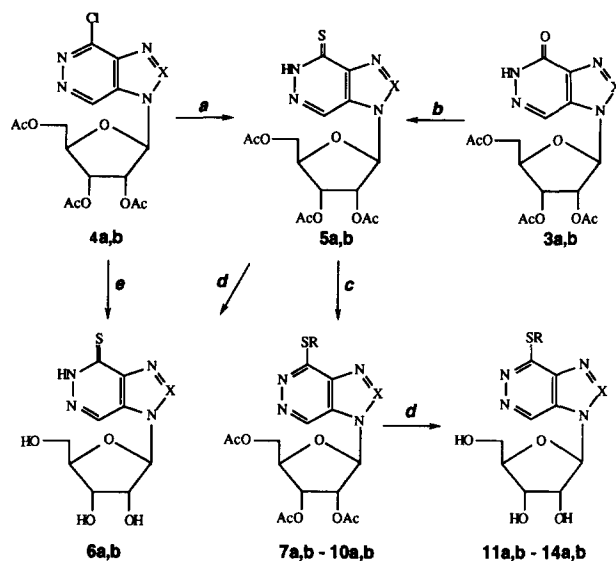


NBTf, 2

11a, X = N
11b, X = CH

pyridazin-4(5*H*)-one^{2b} (**3a**) and 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-one¹⁰ (**3b**) as starting materials. We explored several procedures for the thiation of **3a** and **b**. Although **3a** and **b** are similar in their structural appearance, they differ in their chemical reactivity at the C4 position of the heterocyclic aglycone.^{2b} Therefore, different thiation conditions and precursors were employed for the two series. For instance, displacement reactions involving the respective 4-chloro nucleosides of both series with a variety of nucleophiles occurred more readily with those nucleosides possessing the *v*-triazolo[4,5-*d*]pyridazine ring system.

Direct thiation of **3a** was performed using P₂S₅ and flowers of sulfur in hot dioxane¹¹ to give 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione (**5a**) in moderate yield. An alternate and more efficient route to **5a** involved the use of 4-chloro-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (**4a**).^{2b} Thiation of **4a** was carried out with sodium thiosulfate in a mixture of alcohols and water and the complete mixture was heated to 80 °C to afford the thione **5a** in excellent yield. Although the literature procedure¹² describing this novel thiation method reported concomitant deacetylation of the product, we found that by lowering the reaction temperature deacetylation was prevented. The thiated acetylated product was easily purified due to better organic solubility and obtained in higher yield. Treatment of **5a** with methanolic ammonia furnishes **6a** in good yield. This deacetylated nucleoside, 1-(β -D-ribo-



Scheme 1. In series a, X=N; in series b, X=CH. Reagents: (a) Na₂S₂O₅, BuOH, H₂O, EtOH; (b) P₂S₅ (series a: S₈, dioxane; series b: pyridine); (c) NaH, alkyl halide, THF; (d) MeOH/NH₃; (e) NaSH, MeOH (series a only).

furanosyl)-*v*-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione (**6a**) was also obtained directly from **4a** with the use of sodium hydrosulfide in refluxing methanol.

The thiation of 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-one (**3b**) using P₂S₅ was achieved more readily than in the *v*-triazolo[4,5-*d*]pyridazine nucleoside series (series a). Thiation of **3b** with P₂S₅ in refluxing pyridine¹³ furnished 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine-4(5*H*)-thione (**5b**) in an 87% yield. Likewise, the thione **5b** could also be synthesized through the corresponding chloro nucleoside **4b** (91% yield) using a similar set of reaction conditions described for the preparation of **5a**. In the case of **5b**, however, a longer reaction time was required.

Alkylation of both 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione (**5a**) and 1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine-4(5*H*)-thione (**5b**) was accomplished using the same methodology. A solution of the appropriate nucleoside in dry THF was added dropwise to a suspension of sodium hydride in THF. After 15 min, to ensure the formation of the anion, the alkylating agent was added in excess¹⁴ to give the desired 4-alkylthio derivatives **7a,b**–**10a,b** (Table 1) in yields ranging from of 72–84% depending upon the alkylating agent used. Deprotection also followed a general procedure for both series; the acetylated nucleoside was stirred in saturated methanolic ammonia (satd at –5 °C) for 12 h to give the desired deblocked nucleosides **11a,b**–**14a,b** in good yields.

Biological Evaluation and Discussion

Adenosine enters human erythrocytes by facilitated diffusion via a single, nonspecific carrier system that is

extremely sensitive to inhibition by certain 6-substituted purine nucleosides.⁶ Under the present assay conditions, 0.1 μM incubation concentrations of NBMPR and NBTF cause 99 and 90% inhibition of 10 μM adenosine transport into erythrocytes,¹⁵ respectively. The apparent IC_{50} values for NBMPR and NBTF, with 10% erythrocyte suspensions, are identical, that is, 18 nM.¹⁵ In comparison, **11a** (tested at 0.1–1.0 μM concentration) and **11b** (tested at 0.1–1.0 μM concentration) exhibited IC_{50} values of 500 and 60 nM, respectively. Analogue **13a** caused only 17% inhibition at the highest concentration tested, 10 μM .

On the basis of our results and earlier findings,⁶ the N-3 nitrogen (N-7 in purine numbering) plays a critical role in binding of purine-type nucleoside inhibitors to the transport carrier. It appears that the affinity of these inhibitors for the transporter relies, in part, on a hydrogen bond to the N-7 purine position. In the case of NBMPR, the N-7 nitrogen is a hydrogen bond acceptor, whereas the N(3)H position of NBTF (most likely the predominant tautomer in solution) is a hydrogen bond donor. Although their IC_{50} values are the same (18 nM), the affinity of radiolabeled NBMPR

for the transporter is 10-fold higher than that of radiolabeled NBTF.¹⁵ To a certain extent this difference in binding may be attributed to the type and strength of the hydrogen bond formed with the carrier. The selected ribonucleosides examined as inhibitors in this study are hydrogen bond acceptors. Therefore, the low affinity exhibited by nucleosides **11a** and **13a** may reflect the weakening of this hydrogen bond at N-3 by the adjacent N-2 nitrogen.

Experimental

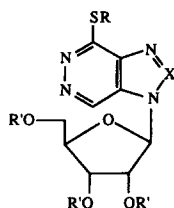
Melting points were determined on a Buchi 535 melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on either a Varian EM 390 or a Bruker AM-300 spectrometer, as indicated, using Me_4Si (TMS) as an int. standard. Optical rotations were measured on a Perkin–Elmer Model 141 automatic digital readout polarimeter. UV absorption spectra were recorded with a Beckman DU-64 spectrophotometer. All moisture-sensitive reactions were performed using flame-dried glassware. Methylene chloride (CH_2Cl_2) and acetonitrile were dried over CaH_2 and distilled. Anhydrous THF was obtained by distillation over sodium benzophenone ketyl. Evapns were performed under diminished pressure using a Buchi Rotary Evaporator unless noted otherwise. Davison silica gel (grade H, 60–200 mesh), purchased from Fisher Scientific, was used for flash column chromatography. Thin-layer chromatography was performed on precoated silica gel plates (60–F254, 0.2 mm) manufactured by E.M. Science, Inc. and short-wave ultraviolet light (254 nm) was used to detect the UV absorbing compounds. All solvent proportions are by volume unless otherwise indicated. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

Synthesis

1-(2,3,5-Tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione (5a**). Method A: Sodium thiosulfate pentahydrate (1.42 g, 5.72 mmol) was added to a soln of **4a**^{2b} (0.48 g, 1.16 mmol) in a mixture containing butanol:water:ethanol (4:1:2, 10 mL). The reaction mixture was heated at 80 $^\circ\text{C}$ for 10 h, cooled to room temperature, and then concd. The crude product was purified by flash column chromatography using CH_2Cl_2 :ethyl acetate (4:1) as the eluent to afford **5a** (0.47 g, 92% yield) as a yellow solid: mp 103–104 $^\circ\text{C}$; ^1H NMR (CDCl_3): δ 1.91 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 4.10–4.65 (m, 3H, H-5', H-5'', H-4'), 5.56 (t, 1H, H-3'), 6.02 (t, 1H, $J_{2',1'}=4.5$ Hz, H-2'), 6.43 (d, 1H, $J_{1,2'}=4.0$ Hz, H-1'), 8.62 (s, 1H, H-7), 12.20 (s, 1H, D_2O exchangeable, NH). Anal. calcd for $\text{C}_{15}\text{H}_{17}\text{N}_5\text{O}_5\text{S}$: C, 43.79; H, 4.17; N, 17.02; S, 7.79. Found: C, 43.55; H, 4.34; N, 16.88; S, 7.56.**

Method B: To a heated soln (ca. 60 $^\circ\text{C}$) of **3a**^{2b} (0.13 g, 0.32 mmol) in dioxane (6 mL) was added P_2S_5 (72 mg, 0.32 mmol) and S_8 (sulfur flowers, 5 mg). The reaction

Table 1. S^4 -analogues



Compound	X	R'	R
5a	N	Ac	H
5b	CH	Ac	
6a	N	H	H
6b	CH	H	
7a	N	Ac	
7b	CH	Ac	
8a	N	Ac	
8b	CH	Ac	
9a	N	Ac	
9b	CH	Ac	
10a	N	Ac	
10b	CH	Ac	
11a	N	H	
11b	CH	H	
12a	N	H	
12b	CH	H	
13a	N	H	
13b	CH	H	
14a	N	H	
14b	CH	H	

was stirred at 60 °C for 25 min and then an additional amount of P_2S_5 (70 mg, 0.31 mmol) was added. After stirring at 60 °C for 30 min more, the reaction mixture was cooled to room temperature and poured over cracked ice. The resulting aq soln was extracted with CH_2Cl_2 (3 × 30 mL) and the organic layers were combined, washed with satd aq $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, filtered, and then concd. The crude product was purified by flash column chromatography using CH_2Cl_2 :ethyl acetate (9:1) as the eluent to afford **5a** (85 mg, 64% yield) as a yellow solid having physical data that was identical in all respects to that prepared in Method A.

1-(2,3,5-Tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]-pyridazine-4(5*H*)-thione (5b). Method A: Compound **4b**^{2b} (0.32 g, 0.78 mmol) was thiated using sodium thiosulfate pentahydrate (1.15 g, 4.63 mmol) by following the procedure described for the preparation of **5a**. The reaction mixture was heated at 80 °C for 16 h, cooled to room temperature and then concd. Purification of the crude product by flash column chromatography using CH_2Cl_2 :ethyl acetate (4:1) as the eluent afforded **5b** (0.29 g, 91% yield) as a yellow foam: mp 103–104 °C; 1H NMR ($CDCl_3$): δ 1.91 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 4.30–4.65 (m, 3H, H-5', H-5'', H-4'), 5.20–5.45 (m, 2H, H-3', H-2'), 6.08 (d, 1H, $J_{1,2'}=4.5$ Hz, H-1'), 8.25 (s, 1H, H-2), 8.63 (s, 1H, H-7), 12.62 (s, 1H, D_2O exchangeable, NH). Anal. calcd for $C_{16}H_{18}N_4O_7S$: C, 46.80; H, 4.41; N, 13.62; S, 7.81. Found: C, 46.70; H, 4.55; N, 13.51; S, 7.71.

Method B: A mixture containing **3b**^{2b} (0.60 g, 1.52 mmol), P_2S_5 (1.50 g, 3.38 mmol), H_2O (3 drops), and pyridine (10 mL) was heated at reflux for 12 h. The reaction mixture was cooled to room temperature and then H_2O (10 mL) was added. The aq soln was extracted with CH_2Cl_2 (2 × 10 mL) and the organic layers were combined, washed with satd aq $NaHCO_3$, dried over $MgSO_4$, filtered and then concd. Purification of the crude product by flash column chromatography using CH_2Cl_2 :ethyl acetate (4:1) as the eluent afforded **5b** (0.55 g, 87% yield) as a yellow foam having physical data identical in all respects to that obtained by Method A.

General deacetylation procedure

1-(β -D-Ribofuranosyl)-v-triazolo[4,5-*d*]pyridazine-4(5*H*)-thione (6a). Method A: A soln of **5a** (0.120 g, 0.29 mmol) and satd methanolic ammonia solution (10 mL, saturated at –5 °C) was stirred for 12 h at room temperature and then the solvents were removed under red. pres. Purification of the residue by flash column chromatography using ethyl acetate:MeOH (99:1) as the eluent, followed by recrystallization of the product from 95% ethanol gave pure **6a** (82 mg, 98% yield) as a yellow solid: mp 169–170 °C; $[\alpha]_D^{24} -41.1^\circ$ (c 0.86, MeOH); 1H NMR (DMSO- d_6 , 300 MHz): δ 3.52–3.67 (m, 2H, H-5', H-5''), 4.09 (q, 1H, $J_{4',5'}=7.57$, $J_{4',3'}=4.28$ Hz, H-4'), 4.22 (q, 1H, $J_{3',2'}=9.87$, $J_{3',4'}=4.96$ Hz, H-3'), 4.61 (q, 1H, $J_{2',3'}=9.78$, $J_{2',1'}=4.93$ Hz, H-2'), 5.02 (t, 1H, $J=5.12$ Hz, D_2O exchangeable, OH), 5.35 (d, 1H,

$J=5.46$ Hz, D_2O exchangeable, OH), 5.72 (d, 1H, $J=5.70$ Hz, D_2O exchangeable, OH), 6.32 (d, 1H, $J_{1,2'}=4.42$ Hz, H-1'), 7.37 (s, 1H, H-7), 14.55 (bs, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6): δ 61.0 (C-5'), 70.3 (C-3'), 74.9 (C-2'), 86.7 (C-4'), 93.0 (C-1'), 106.6 (C-7), 127.6 (C-7a), 131.2 (C-3a), 176.5 (C-4). Anal. calcd for $C_9H_{11}N_5O_4S$: C, 37.89; H, 3.89; N, 24.55; S, 11.24. Found: C, 37.69; H, 4.00; N, 24.39; S, 11.39.

Method B: In an alternate synthesis of **6a**, a soln containing **4a**^{2b} (0.14 g, 0.33 mmol) and 1 N sodium hydrosulfide (5 mL) was heated at 60 °C overnight in a glass-lined steel reaction vessel. The reaction mixture was cooled to room temperature, the residue dissolved in 95% ethanol and then acidified to pH 6 with 1.7 M aq acetic acid. The yellow precipitate was collected by filtration and recrystallized from 95% ethanol to give **6a** (70.6 mg, 74% yield) having physical data identical in all respects to that obtained by Method A.

1-(β -D-Ribofuranosyl)imidazo[4,5-*d*]pyridazine-4(5*H*)-thione (6b). Compound **6b** (0.50 g, 1.22 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual work up followed by recrystallization from 95% ethanol gave **6b** (0.27 g, 81% yield) as a yellow solid: mp 232–233 °C; $[\alpha]_D^{25} -66.1^\circ$ (c 1.31, DMF); 1H NMR (DMSO- d_6 , 300 MHz): δ 3.65–3.74 (m, 2H, H-5', H-5''), 4.06–4.09 (m, 1H, H-4'), 4.14–4.17 (m, 1H, H-3'), 4.28 (t, 1H, $J_{2',1'}=6.00$ Hz, H-2'), 5.98 (d, 1H, $J_{1,2'}=6.26$ Hz, H-1'), 8.72 (s, 1H, H-2), 9.08 (s, 1H, H-7), 14.32 (bs, 1H, D_2O exchangeable, NH) [lit.¹³ 1H NMR (DMSO- d_6 , 100 MHz): δ 3.67 (m, 2H, H-5', H-5''), 4.0–4.3 (m, 3H, H-2', H-3', H-4'), 5.95 (d, 1H, $J=6.1$ Hz, H-1'), 8.69 (s, 1H, H-2), 9.05 (s, 1H, H-7), 14.31 (bs, 1H, NH)]. Anal. calcd for $C_{10}H_{12}N_4O_4S$: C, 42.13; H, 4.53; N, 19.65; S, 11.25. Found: C, 41.97; H, 4.42; N, 19.91; S, 11.13.

General alkylation procedure

4-*p*-Nitrobenzylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-v-triazolo[4,5-*d*]pyridazine (7a). To a suspension of NaH (67 mg, 1.69 mmol, 60% dispersion in mineral oil) in dry THF (5 mL) was added, a soln of **5a** (0.59 g, 1.41 mmol) in dry THF (5 mL) under nitrogen at room temperature. After stirring for 15 min, *p*-nitrobenzyl bromide (0.39 g, 1.81 mmol) was added and the reaction mixture was stirred for 3 h. The solvent was removed under diminished pressure and the residue purified by flash column chromatography using CH_2Cl_2 :ethyl acetate (4:1) as the eluent to furnish **7a** (0.33 g, 72% yield) as a yellow foam: mp 75–76 °C; 1H NMR ($CDCl_3$, 300 MHz): δ 1.98 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.22–4.39 (m, 2H, H-5', H-5''), 4.53–4.60 (m, 1H, H-4'), 4.83 (s, 2H, $CH_2C_6H_4NO_2$), 5.60 (t, 1H, $J_{2',1'}=4.5$ Hz, H-3') 6.02 (t, 1H, $J_{2',1'}=4.5$ Hz, H-2'), 6.47 (d, 1H, $J_{1,2'}=4.4$ Hz, H-1'), 7.70 (ABq, 2H, $J_{AB}=8.7$ Hz, $CH_2C_6H_4NO_2$), 8.14 (ABq, 2H, $J_{AB}=8.7$ Hz, $CH_2C_6H_4NO_2$), 9.49 (s, 1H, H-7). Anal. calcd for $C_{22}H_{22}N_6O_6S$: C, 48.35; H, 4.06;

N, 15.38; S, 5.87. Found: C, 48.31; H, 4.12; N, 15.20; S, 5.74.

4-*p*-Nitrobenzylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine (7b). The prep and purification of **7b** was carried out as for the synthesis of **7a**. Alkylation of **5b** (0.42 g, 1.02 mmol) using *p*-nitrobenzyl bromide (0.44 g, 2.03 mmol) afforded **7b** (0.42 g, 76% yield) as a yellow foam: mp 64–65 °C; ¹H NMR (CDCl₃): δ 1.99 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.14 (s, 3H, CH₃), 4.32–4.58 (m, 3H, H-5', H-5'', H-4'), 4.75 (s, 2H, CH₂C₆H₄NO₂), 5.18–5.48 (m, 2H, H-3', H-2') 6.06 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 7.61 (ABq, 2H, $J_{AB}=8.5$ Hz, CH₂C₆H₄NO₂), 8.05 (ABq, 2H, $J_{AB}=8.5$ Hz, CH₂C₆H₄NO₂), 8.23 (s, 1H, H-2), 9.37 (s, 1H, H-7). Anal. calcd for C₂₃H₂₃N₅O₉S: C, 50.64; H, 4.25; N, 12.84; S, 5.88. Found: C, 50.85; H, 4.43; N, 12.74; S, 6.06.

4-Methylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (8a). The preparation and purification of **8a** was carried out as for the synthesis of **7a**. Alkylation of **5a** (0.59 g, 1.41 mmol) using methyl iodide (0.10 mL, 1.61 mmol) afforded pure **8a** (0.33 g, 73% yield) as a yellow foam: mp 62–63 °C; ¹H NMR (CDCl₃): δ 1.88 (s, 3H, CH₃), 1.98 (s, 3H, CH₃), 2.20 (s, 3H, CH₃), 2.85 (s, 3H, SCH₃), 4.18–4.45 (m, 2H, H-5', H-5''), 4.53–4.58 (m, 1H, H-4'), 5.28–5.58 (m, 1H, H-3'), 5.43 (t, 1H, $J_{2',1'}=4.5$ Hz, H-2'), 6.35 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 9.45 (s, 1H, H-7). Anal. calcd for C₁₆H₁₉N₅O₇S: C, 45.17; H, 4.50; N, 16.46; S, 7.54. Found: C, 45.12; H, 5.13; N, 16.41; S, 7.59.

4-Methylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-imidazo[4,5-*d*]pyridazine (8b). The preparation and purification of **8b** was carried out as for the synthesis of **7a**. Alkylation of **5b** (0.60 g, 1.46 mmol) using methyl iodide (0.11 mL, 1.76 mmol) afforded **8b** (0.45 g, 73% yield) as a yellow foam: mp 60–61 °C; ¹H NMR (CDCl₃): δ 2.07 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.18 (s, 3H, CH₃), 2.81 (s, 3H, CH₃), 4.32–4.58 (m, 3H, H-5', H-5'', H-4'), 5.28–5.50 (m, 2H, H-3', H-2'), 6.13 (d, 1H, $J_{1',2'}=5.0$ Hz, H-1'), 8.29 (s, 1H, H-2), 9.39 (s, 1H, H-7). Anal. calcd for C₁₇H₂₀N₄O₇S: C, 48.11; H, 4.75; N, 13.20. Found: C, 48.16; H, 4.91; N, 13.07.

4-[(1-Methyl-4-nitroimidazol-5-yl)thio]-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (9a). The preparation and purification of **9a** was carried out as for the synthesis of **7a**. Alkylation of **5a** (0.31 g, 0.76 mmol) using 5-chloro-1-methyl-4-nitroimidazole (0.12 g, 0.80 mmol) afforded **9a** (0.32 g, 80% yield) as a yellow solid: mp 176–177 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.98 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.14 (s, 3H, CH₃), 3.80 (s, 3H, CH₃), 4.25–4.40 (dq, 2H, H-5', H-5''), 4.57–4.59 (m, 1H, H-4'), 5.59 (t, 1H, $J=5.4$ Hz, H-3'), 6.04 (t, 1H, $J_{2',1'}=4.5$ Hz, H-2'), 6.49 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 7.76 (s, 1H, imidazole-CH), 9.55 (s, 1H, H-7). Anal. calcd for C₁₉H₂₀N₈O₉S: C, 42.54; H, 3.76; N, 20.89; S, 5.98. Found: C, 42.39; H, 4.00; N, 20.84; S, 6.11.

4-[(1-Methyl-4-nitroimidazol-5-yl)thio]-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine (9b). The preparation and purification of **9b** was carried out as for the synthesis of **7a**. Alkylation of **5b** (0.33 g, 0.80 mmol) using 5-chloro-1-methyl-4-nitroimidazole (0.26 g, 1.60 mmol) afforded pure **9b** (0.32 g, 73% yield) as a yellow foam: mp 113–115 °C; ¹H NMR (CDCl₃): δ 2.10 (s, 3H, CH₃), 2.16 (s, 3H, CH₃), 2.18 (s, 3H, CH₃), 3.79 (s, 3H, CH₃), 4.23–4.63 (m, 3H, H-5', H-5'', H-4'), 5.23–5.57 (m, 2H, H-3', H-2'), 6.18 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 7.71 (s, 1H, imidazole-CH), 8.34 (s, 1H, H-2) 9.45 (s, 1H, H-7). Anal. calcd for C₂₀H₂₁N₇O₉S: C, 44.86; H, 3.95; N, 18.31; S, 5.99. Found: C, 45.08; H, 3.95; N, 18.18; S, 5.61.

4-Benzylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (10a). The preparation and purification of **10a** was carried out in the same manner as for **7a**. Alkylation of **5a** (0.24 g, 0.58 mmol) using benzyl bromide (0.14 mL, 1.17 mmol) afforded pure **10a** (0.22 g, 75% yield) as a yellow foam: mp 53–54 °C; ¹H NMR (CDCl₃): δ 1.97 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 4.13–4.63 (m, 3H, H-5', H-5'', H-4''), 4.79 (s, 2H, CH₂C₆H₅), 5.58 (t, 1H, $J=5$ Hz, H-3'), 6.02 (t, 1H, $J_{2',1'}=4.5$ Hz, H-2'), 6.43 (d, 1H, $J_{1',2'}=4$ Hz, H-1'), 7.17–7.57 (m, 5H, C₆H₅), 9.48 (s, 1H, H-7). Anal. calcd for C₂₂H₂₃N₅O₇S: C, 52.69; H, 4.62; N, 13.96; S, 6.39. Found: C, 52.50; H, 4.70; N, 13.75; S, 6.18.

4-Benzylthio-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-imidazo[4,5-*d*]pyridazine (10b). The preparation and purification of **10b** was carried out as for the synthesis of **7a**. Alkylation of **5b** (0.69 g, 1.68 mmol) using benzyl bromide (0.24 mL, 2.02 mmol) afforded **10b** (0.70 g, 84% yield) as a yellow foam: mp 55–57 °C; ¹H NMR (CDCl₃): δ 2.06 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.16 (s, 3H, CH₃), 4.21–4.58 (m, 3H, H-5', H-5'', H-4'), 4.73 (s, 2H, CH₂C₆H₅), 5.24–5.51 (m, 2H, H-3', H-2'), 6.09 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 7.08–7.54 (m, 5H, C₆H₅), 8.22 (s, 1H, H-2), 9.39 (s, 1H, H-7). Anal. calcd for C₂₃H₂₄N₄O₇S: C, 55.19; H, 4.83; N, 11.19; S, 6.41. Found: C, 55.52; H, 5.01; N, 10.86; S, 6.22.

4-*p*-Nitrobenzylthio-1-(β -D-ribofuranosyl)-*v*-triazolo[4,5-*d*]pyridazine (11a). Compound **7a** (120 mg, 0.21 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual workup, followed by crystallization of the product from 95% ethanol afforded pure **11a** (77 mg, 88% yield) as a yellow solid: mp 103–104 °C; $[\alpha]_D^{25} -58.2^\circ$ (c 1.08, EtOH); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 3.58 (dq, 2H, $J_{5',5''}=12.3$, $J_{4',5'}=3.9$ Hz, H-5', H-5''), 4.08 (dd, 1H, $J_{4',5'}=3.9$ Hz, H-4'), 4.21 (t, 1H, $J=4.8$ Hz, H-3'), 4.63 (t, 1H, $J_{2',1'}=4.8$ Hz, H-2'), 4.90 (s, 2H, CH₂C₆H₄NO₂), 5.00 (bs, 1H, D₂O exchangeable, OH), 5.31 (bs, 1H, D₂O exchangeable, OH), 5.64 (bs, 1H, D₂O exchangeable, OH), 6.39 (d, 1H, $J_{1',2'}=4.8$ Hz, H-1') 7.77 (ABq, 2H, $J_{AB}=8.7$ Hz, CH₂C₆H₄NO₂), 8.14 (ABq, 2H, $J_{AB}=8.7$ Hz, CH₂C₆H₄NO₂), 9.93 (s, 1H, H-7); ¹³C NMR (DMSO-*d*₆): δ 31.2, 61.2, 70.4, 74.8, 86.9, 93.1, 123.9, 129.0, 130.8, 137.7, 140.6, 146.3, 147.1,

154.3, 172.4. Anal. calcd for $C_{16}H_{16}N_6O_6S$: C, 45.71; H, 3.84; N, 19.99; S, 7.63. Found: C, 45.88; H, 4.00; N, 19.74; S, 7.44.

4-*p*-Nitrobenzylthio-1-(β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine (11b). Compound **7b** (0.36 g, 6.70 mmol) was deacetylated following the procedure described for the preparation of **6a** (Method A). The usual work up followed by crystallization from 95% ethanol gave pure **11b** (0.22 g, 80% yield) as a yellow solid: mp 102–103 °C; $[\alpha]_D^{25} -54.2^\circ$ (*c* 0.5, DMF); 1H NMR (DMSO-*d*₆, 300 MHz): δ 3.62–3.72 (m, 2H, H-5', H-5''), 4.04–4.10 (m, 1H, H-4'), 4.13–4.17 (m, 1H, H-3'), 4.32 (q, 1H, $J_{2',1'}=6.24$ Hz, H-2'), 4.85 (s, 2H, $CH_2C_6H_4NO_2$), 5.30 (t, 1H, $J=4.9$ Hz, D₂O exchangeable OH), 5.34 (d, 1H, $J=4.4$ Hz, D₂O exchangeable OH), 5.60 (d, 1H, $J=6.35$ Hz, D₂O exchangeable OH), 6.02 (d, 1H, $J_{1',2'}=6.4$ Hz, H-1') 7.77 (ABq, 2H, $J_{AB}=8.7$ Hz, $CH_2C_6H_4NO_2$), 8.16 (ABq, 2H, $J_{AB}=8.7$ Hz, $CH_2C_6H_4NO_2$), 8.81 (s, 1H, H-2) 9.71 (s, 1H, H-7); ^{13}C NMR (DMSO-*d*₆): δ 31.0, 61.1, 70.2, 74.9, 86.5, 89.7, 123.4, 128.8, 130.2, 137.2, 138.9, 145.2, 146.5, 146.6, 152.9. Anal. calcd for $C_{17}H_{17}N_5O_6S$: C, 48.68; H, 4.09; N, 16.70; S, 7.64. Found: C, 48.61; H, 4.09; N, 16.72; S, 7.57.

4-Methylthio-1-(β -D-ribofuranosyl)-v-triazolo[4,5-*d*]pyridazine (12a). Compound **8a** (0.20 g, 0.48 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual reaction work up gave **12a** (0.11 g, 75% yield) as a yellow solid: mp 154–155 °C; $[\alpha]_D^{25} -26.6^\circ$ (*c* 2.62, MeOH); 1H NMR (DMSO-*d*₆, 300 MHz): δ 2.81 (s, 3H, CH_3), 3.53–3.68 (m, 2H, H-5', H-5''), 4.10 (q, 1H, $J=7.3$, $J=3.8$ Hz, H-4'), 4.24 (q, 1H, $J=9.6$, $J=4.9$ Hz, H-3'), 4.65 (q, 1H, $J=10.4$, $J_{2',1'}=5.1$ Hz, H-2'), 5.06 (t, 1H, $J=5.0$ Hz, D₂O exchangeable OH), 5.36 (d, 1H, $J=5.3$ Hz, D₂O exchangeable OH), 5.70 (d, 1H, $J=5.8$ Hz, D₂O exchangeable OH), 6.42 (d, 1H, $J_{1',2'}=4.8$ Hz, H-1'), 9.94 (s, 1H, H-7); ^{13}C NMR (DMSO-*d*₆): δ 11.6, 61.0, 70.2, 74.5, 86.6, 92.7, 128.2, 136.6, 140.3, 155.4. Anal. calcd for $C_{10}H_{13}N_5O_4S$: C, 40.13; H, 4.38; N, 23.40; S, 10.71. Found: C, 39.68; H, 4.26; N, 22.96; S, 10.66.

4-Methylthio-1-(β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine (12b). Compound **8b** (0.28 g, 0.65 mmol) was deacetylated by following the procedure for the preparation of **6a** (Method A). The usual work up followed by crystallization from 95% ethanol gave pure **12b** (0.15 g, 81% yield) as a yellow solid: mp 177–178 °C; $[\alpha]_D^{25} -61.3^\circ$ (*c* 0.79, DMF); 1H NMR (DMSO-*d*₆, 300 MHz): δ 2.72 (s, 3H, CH_3), 3.63–3.75 (m, 2H, H-5'), 4.02–4.07 (m, 1H, H-4'), 4.11–4.18 (m, 1H, H-3'), 4.32 (t, 1H, $J_{2',1'}=6.12$ Hz, H-2'), 5.28–5.37 (m, 2H, D₂O exchangeable OH), 5.57–5.65 (m, 1H, D₂O exchangeable OH), 6.01 (d, 1H, $J_{1',2'}=6.48$ Hz, H-1'), 8.77 (s, 1H, H-2), 9.66 (s, 1H, H-7) [Lit.¹³ 1H NMR (DMSO-*d*₆, 100 MHz): δ 2.73 (s, 3H, SCH_3), 3.68 (m, 2H, H-5', H-5''), 4.0–4.2 (m, 2H, H-3', H-4'), 4.31 (m, 1H, H-2'), 6.01 (d, 1H, $J=6.1$ Hz, H-1'), 8.77 (s, 1H, H-2), 9.67 (s, 1H, H-7)]; ^{13}C NMR (DMSO-*d*₆):

δ 11.5, 61.1, 70.2, 74.8, 86.5, 89.6, 128.6, 136.7, 139.0, 144.9, 154.6. Anal. calcd for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 44.23; H, 4.77; N, 18.68; S, 10.65.

4-[(1-Methyl-4-nitroimidazol-5-yl)thio-1-(β -D-ribofuranosyl)-v-triazolo[4,5-*d*]pyridazine (13a). Compound **9a** (0.20 g, 0.39 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual reaction work up gave **13a** (0.13 g, 80% yield) as a yellow solid: mp 183–185 °C; $[\alpha]_D^{25} -61.2^\circ$ (*c* 1.08, EtOH); 1H NMR (DMSO-*d*₆, 300 MHz): δ 3.50–3.65 (m, 2H, H-5', H-5''), 3.73 (s, 3H, CH_3), 4.07–4.09 (m, 1H, H-4'), 4.19–4.22 (m, 1H, H-3'), 4.64 (q, 1H, H-2'), 5.03 (t, 1H, $J=4.8$ Hz, D₂O exchangeable OH), 5.35 (d, 1H, $J=5.1$ Hz, D₂O exchangeable OH), 5.70 (d, 1H, $J=5.7$ Hz, D₂O exchangeable OH), 6.41 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1'), 8.28 (s, 1H, imidazole-CH), 10.33 (s, 1H, H-7); ^{13}C NMR (DMSO-*d*₆): δ 33.2, 61.1, 70.4, 74.8, 87.0, 93.2, 116.7, 129.9, 138.9, 140.2, 140.3, 150.2, 153.2. Anal. calcd for $C_{13}H_{14}N_8O_6S$: C, 38.05; H, 3.44; N, 27.31; S, 7.81. Found: C, 38.28; H, 3.66; N, 27.12; S, 7.63.

4-[(1-Methyl-4-nitroimidazol-5-yl)thio]-1-(β -D-ribofuranosyl)imidazo[4,5-*d*]pyridazine (13b). Compound **9b** (0.25 g, 0.47 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual work up gave **13b** (0.16 g, 82% yield) as a yellow solid: mp 176–177 °C; $[\alpha]_D^{25} -54.7^\circ$ (*c* 0.96, DMF); 1H NMR (DMSO-*d*₆, 300 MHz): δ 3.68–3.71 (m, 5H, H-5', H-5'', CH_3), 4.05 (m, 1H, H-4'), 4.13 (q, 1H, H-3'), 4.31 (q, 1H, H-2'), 5.29 (t, 1H, $J=4.9$ Hz, D₂O exchangeable OH), 5.32 (d, 1H, $J=4.38$ Hz, D₂O exchangeable OH), 5.60 (d, 1H, $J=6.26$ Hz, D₂O exchangeable OH), 6.02 (d, 1H, $J_{1',2'}=6.39$ Hz, H-1'), 8.25 (s, 1H, imidazole-CH), 8.87 (s, 1H, H-2), 9.74 (s, 1H, H-7); ^{13}C NMR (DMSO-*d*₆): δ 33.0, 61.0, 70.2, 74.9, 86.6, 89.9, 117.8, 130.1, 138.1, 138.8, 139.4, 146.0, 149.6, 151.5. Anal. calcd for $C_{14}H_{15}N_7O_6S$: C, 41.08; H, 3.69; N, 23.95; S, 7.83. Found: C, 40.70; H, 3.92; N, 23.65; S, 7.93.

4-Benzylthio-1-(β -D-ribofuranosyl)-v-triazolo[4,5-*d*]pyridazine (14a). Compound **10a** (0.20 g, 0.48 mmol) was deacetylated by following the same procedure as for the preparation of **6a** (method A). The usual workup followed by crystallization from $CHCl_3$ gave pure **14a** (0.11 g, 75% yield) as a yellow solid: mp 154–155 °C; $[\alpha]_D^{25} -43.0^\circ$ (*c* 0.73, MeOH); 1H NMR (DMSO-*d*₆, 300 MHz): δ 3.52–3.70 (m, 2H, H-5', H-5''), 4.10 (q, 1H, $J=3.92$ Hz, H-4'), 4.24 (q, 1H, $J=4.87$ Hz, H-3'), 4.66 (q, 1H, $J=4.98$ Hz, H-2'), 4.79 (d, 2H, $CH_2C_6H_5$), 5.05 (t, 1H, $J=5.08$ Hz, D₂O exchangeable OH), 5.36 (d, 1H, $J=5.40$ Hz, D₂O exchangeable OH), 5.69 (d, 1H, $J=5.78$ Hz, D₂O exchangeable OH), 6.42 (d, 1H, $J_{1',2'}=4.75$ Hz, H-1'), 7.25–7.35 (m, 3H, C_6H_5), 7.50–7.53 (m, 2H, C_6H_5), 9.96 (s, 1H, H-7); ^{13}C NMR (DMSO-*d*₆): δ 32.1, 61.0, 70.1, 74.5, 86.6, 92.7, 127.3, 127.8, 128.4, 129.0, 136.9, 137.1, 140.0, 154.5. Anal. calcd for $C_{16}H_{17}N_5O_4S \cdot H_2O$: C,

48.85; H, 4.88; N, 17.80; S, 8.15. Found: C, 48.83; H, 4.73; N, 17.79; S, 8.23.

4-Benzylthio-1-(β -D-ribofuranosyl)imidazo[4,5-d]pyridazine (14b). Compound **10b** (0.33 g, 0.66 mmol) was deacetylated by following the procedure described for the preparation of **6a** (Method A). The usual work up followed by crystallization from 95% ethanol gave pure **14b** (0.21 g, 82% yield) as a yellow solid: mp 205–206 °C; $[\alpha]_D^{25}$ –58.3° (c 0.93, DMF); ^1H NMR (DMSO- d_6 , 300 MHz): δ 3.63–3.76 (m, 2H, H-5', H-5''), 4.02–4.09 (m, 1H, H-4'), 4.11–4.19 (m, 1H, H-3'), 4.28–4.37 (m, 1H, H-2'), 4.71 (bs, 2H, $\text{CH}_2\text{C}_6\text{H}_5$), 5.24–5.35 (m, 2H, D_2O exchangeable, OH), 5.58 (d, 1H, $J=6.05$ Hz, D_2O exchangeable, OH), 6.01 (d, 1H, $J_{1',2'}=6.18$ Hz, H-1'), 7.22–7.32 (m, 3H, C_6H_5), 7.46–7.48 (m, 2H, C_6H_5), 8.78 (s, 1H, H-2), 9.69 (s, 1H, H-7); ^{13}C NMR (DMSO- d_6): δ 31.9, 61.0, 70.1, 74.8, 86.5, 89.7, 127.1, 128.4, 128.9, 129.0, 137.0, 137.9, 138.8, 145.1, 153.8. Anal. calcd for $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_4\text{S}$: C, 54.53; H, 4.85; N, 14.96; S, 8.56. Found: C, 54.44; H, 4.75; N, 14.85; S, 8.39.

Inhibition of nucleoside transport

Nucleoside transport into human erythrocytes was measured at room temperature by an inhibitor-stop assay,¹⁶ with [8- ^{14}C]adenosine (spec. act., 56 mCi/mmol; Moravsek Biochemicals, La Brea, CA) used as the labeled permeant. Analogues were preincubated with triplicate 100 μL cell suspensions and transport was initiated by addition of 100 μL permeant solution to give final concentrations of 10% cells (v/v) and 10 μM adenosine. Transport was terminated after 0.5 s by addition of an equal volume of ice-cold 'stopping solution' containing 750 μM dilazep, a potent transport inhibitor, and centrifugation through oil. Radioactivity was measured after solubilizing and decolorizing the cells. Corrections for extracellular trapping of permeant in the cell pellets were determined from samples to which stopping solution was added before the permeant. The K_m for adenosine transport is 25 ± 14 μM at 22 °C.¹⁷ Apparent IC_{50} values were estimated by use of the ENZFITTER computer program (Elsevier-Biosoft).

Acknowledgments

We thank Dr V. P. Pragnacharyulu and Mrs Rena Fullerton for technical assistance. We also thank Dr M. Gudenzi (Asta-Werke, AG, Frankfurt, F.D.R.) for the gift of the transport inhibitor dilazep.

References and Notes

1. Presented in part at the 201st National Meeting of the American Chemical Society, Atlanta, Georgia, April 1991, MEDI 143.
2. (a) Chen, S.-F.; Panzica, R. P. *J. Heterocycl. Chem.* **1982**, *19*, 285; (b) Bussolari, J. C.; Kakarla, R.; Stoeckler, J. D.; Chen, S.-F.; Panzica, R. P. *J. Med. Chem.* **1993**, *36*, 4113.
3. (a) Chen, S.-F.; Panzica, R. P. *J. Heterocycl. Chem.* **1981**, *18*, 303; (b) Graves, B. J.; Hodgson, D. J.; Chen, S.-F.; Panzica, R. P. *Heterocycles*, **1981**, *16*, 9; (c) Chen, S.-F.; Panzica, R. P. *J. Org. Chem.* **1981**, *46*, 2467.
4. Naguib, F. N. M.; Iltzsch, M. H.; el Kouni, M. M.; Panzica, R. P.; el Kouni, M. H. *Biochem. Pharmacol.* **1995**, *50*, 1685.
5. Belt, J. A.; Marina, N. M.; Phelps, D. A.; Crawford, C. R. *Adv. Enzyme Regul.* **1993**, *33*, 235.
6. Paul, B.; Chen, M.F.; Paterson A.R.P. *J. Med. Chem.* **1975**, *18*, 968.
7. Stoeckler, J. D.; Rosenfield, C. G.; Chu, S.-H.; Li, S.-Y.; Acton, E. M.; Ryan, K. J.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1990**, *40*, 615.
8. Elion, G. B. *Fed. Proc.* **1967**, *26*, 898.
9. Benedict, W. F.; Baker, L.; Haroun, L.; Choi, E.; Ames, B. N. *Cancer Res.* **1977**, *37*, 2209.
10. Kakarla, R.; Panzica, R. P. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1769.
11. Montero, J.-L. G.; Bhat, G. A.; Panzica, R.P.; Townsend, L.B. *J. Heterocycl. Chem.* **1977**, *14*, 483.
12. Jankowski, A. J.; Wise, D.S., Jr.; Townsend, L. B. *Nucleosides Nucleotides* **1989**, *8*, 339.
13. Gagnier, R. P.; Halat, M. J.; Otter, B. A. *J. Heterocycl. Chem.* **1984**, *21*, 481.
14. Although we experienced dialkylation^{3c} of the imidazo[4,5-d]pyridazine ring, per se, in the presence of excess alkylating agent, we found this not to be the case when this ring system was substituted on the N-1 of the five-membered ring portion of this heterocycle. This also holds true for the v-triazolo[4,5-d]pyridazine ring system.
15. Stoeckler, J. D.; Rosenfield, C. G.; Chu, S.-H.; Li, S.-Y.; Acton, E. M.; Ryan, K. J.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1990**, *40*, 615.
16. Chen, S.-F.; Cleaveland, J. C.; Hollmann, A.; Weimann, M. C.; Parks, R. E., Jr.; Stoeckler, J. D. *Cancer Res.* **1986**, *46*, 3449.
17. Paterson, A. R. P.; Harley, E. R.; Cass, C. E. *Biochem. J.* **1984**, *224*, 1001.

(Received in U.S.A. 15 April 1996; accepted 28 June 1996)