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EFFECTS OF CHEMICAL MODIFICATION OF NITROBENZYLTHIOINOSINE ON ITS BINDING TO HIGH-AFFINITY MEMBRANE BINDING SITES AND INHIBITION OF NUCLEOSIDE TRANSPORT

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Abstract—Nitrobenzylthioinosine (NBTI) was systematically modified by attachment of substituents at the 2-, 5′-, 3′- and 2′-positions in order to assess the importance of these positions in the binding of NBTI to high-affinity membrane binding sites ($K_d \leq 1$ nM) and the inhibition of NBTI-sensitive, equilibrative nucleoside transport by mammalian cells. We determined the effect of the derivatives on the equilibrium binding of 1 nM [^{3}H]NBTI to human erythrocytes and mouse P388 leukemia cells and on the inhibition of zero-*trans* influx of formycin B in P388 cells and equilibrium exchange of uridine in human erythrocytes. Placement of substituent groups at the 5′-position of NBTI had relatively little effect on its binding to high-affinity binding sites or its inhibition of nucleoside transport, regardless of the size of the substituent group (up to about 1000 kDa). All substituents at the 2-position considerably reduced the affinity of NBTI to membrane binding sites and its potency as an inhibitor of nucleoside transport, but some substituent groups reduced the affinity of binding more than the inhibition of nucleoside transport. The effect of the 2-substituents was not directly related to their size. Attachment of a succinate at the 3′- or 5′-position also reduced to a greater extent the binding of NBTI than its inhibition of nucleoside transport, which was relatively little affected. Attachment of succinates at both the 3′ and 5′-positions almost completely abolished both binding to high-affinity sites and inhibition of nucleoside transport. Both functions of NBTI were abolished completely by the simultaneous blockage of the 2′- and 3′-positions. None of the NBTI derivatives significantly inhibited NBTI-resistant equilibrative formycin B transport in P388 and Novikoff rat hepatoma cells at concentrations of ≤ 1 μM.

Nitrobenzylthioinosine (NBTI‖) is a potent inhibitor of one form of equilibrative nucleoside transport (designated NBTI-sensitive) in mammalian cells (reviewed in Ref. 1). Inhibition of this carrier results from binding of NBTI to high-affinity membrane sites ($K_d \sim 1$ nM) which seem to be composed of the substrate binding site of the carrier and a hydrophobic domain that is associated with the carrier and seems to accommodate the interaction with the lipophilic nitrobenzyl group of NBTI [1]. Both structural consideration and experimental evidence indicate that the latter group is responsible for the approximately 10^6-fold higher affinity of NBTI to the transporter as compared to that of the natural nucleosides that are substrates for the carrier [1–4]. Another form of equilibrative nucleoside transporter of mammalian cells is not part of high-affinity NBTI-binding sites and is thus highly resistant to inhibition by NBTI [1, 5, 6] as are the Na^+-dependent, concentrative nucleoside transporters of mammalian cells [1, 7].

Nothing is presently known about the nature of the hydrophobic domain of the high-affinity NBTI-binding site, the nature of its association with the nucleoside carrier and the chemical basis of the interaction of NBTI with the two components of the high-affinity binding site, the substrate binding site of the nucleoside carrier and the hydrophobic domain. An earlier study [3] indicated that the NO_2 group at the benzyl-4 position of NBTI and nitrobenzylthioguanosine (NBTG) and the position of the NO_2 group in the benzyl ring are prime factors in determining the potency of inhibition of nucleoside transport in human erythrocytes. For example, NBTG inhibited nucleoside transport about 300, 200 and 10 times more effectively than 6-(benzylthio) Guo, 6-[(2-nitrobenzyl)thio]Guo and 6-[(3-nitrobenzyl)thio]Guo, respectively. To gain further information on the interaction of NBTI with the nucleoside transporter-associated binding site we systematically placed a number of substituent groups at the purine-2 and ribose 3′- and 5′-positions of NBTI (see Fig. 1) and determined to what extent these modifications affected both its binding to the high-affinity NBTI-binding site and its inhibition of NBTI-sensitive, equilibrative nucleoside transport.

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‖ Abbreviations: NBTI, 6-[(4-nitrobenzyl)thio]inosine; NBTG, 6-[(4-nitrobenzyl)thio]guanosine; Guo, guanosine; and DMF, dimethylformamide.

MATERIALS AND METHODS

Synthesis of NBTI derivatives (see Fig. 1)

A-1, A-2, A-7, A-8 and *A-9* were prepared by coupling of 2-fluoro-6-(4-nitrobenzyl)thio-9-β-D-

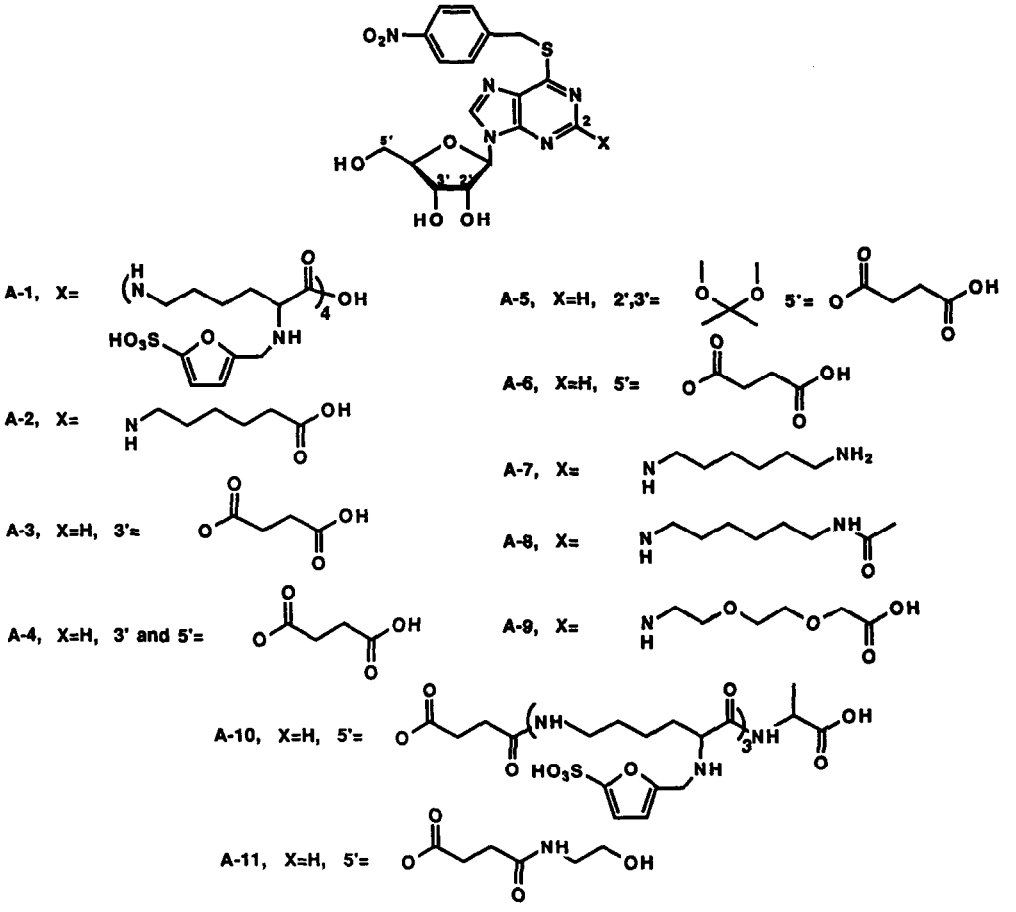

Fig. 1. Structure of 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBTI) and various derivatives thereof possessing the following substituent groups: *A-1*, 2-α-*N*-[(5-sulfonate-2-furanyl)methyl]-lysine tetramer; *A-2*, 2-(6-amino)caproic acid; *A-3*, 3'-succinate; *A-4*, 3',5'-disuccinate; *A-5*, 2',3'-isopropylidene-5'-succinate; *A-6*, 5'-succinate; *A-7*, 2-hexyldiamine; *A-8*, 2-*N*-acetylhexyldiamine; *A-9*, 2-aminoethoxyethoxyacetic acid; *A-10*, 5'-{α-*N*-[(5-sulfonate-2-furanyl)methyl]-lysine trimer-alanine}succinate; and *A-11*, 5'-(*N*-ethanolamine)-succinate.

ribofluranosylpurine [8] with the appropriate amine as described by Kawana *et al.* [9]. Products were isolated and purified by HPLC and analyzed by mass spectrometry and nuclear magnetic resonance analysis.

A-3, A-4, A-5, A-6, A-10, *and* A-11. NBTI succinates were prepared by esterification of NBTI or the NBTI-2',3'-*O*-isopropylidene derivative [10] with succinic anhydride using a modification of the procedure of Matsuda *et al.* [11] [dimethylformamide (DMF) at 50° for 24 hr]. Products were purified by reverse phase HPLC on a C-18 column using a methanol/water solvent system. The 2',3'-*O*-isopropylidene protecting group for *A-6*, *A-10* and *A-11* was removed [12] at the last step.

Amine precursor of A-1 *and* A-10. Synthesis of α-*N*-[(5-sulfonate-2-furanyl)methyl]lysine-tetramer or -trimer alanine was performed on an Applied Biosystems 431A peptide synthesizer using α-tBoc-ε-fmoc-L-lysine and *p*-hydroxymethyl-phenoxymethyl resin. Purification of each product was carried out by reverse phase HPLC on a C-18 column using a water-0.1% trifluoroacetic acid/acetonitrile solvent system. The synthesis was completed without removal of the ε-fmoc protecting group, and the peptide was cleaved from the resin. An aqueous suspension of 5-formyl-2-furanosulfonic acid sodium salt was added to the peptide in a methanol/water solution and the acidity was adjusted to pH6. Aqueous sodium cyanoborohydride was added and the reaction was allowed to proceed for 20 min. The fmoc protecting group was removed with 5% piperidine/DMF solution for 10 min and the product purified by HPLC.

The aminoethoxyethoxyacetic acid spacer for *A-9* was prepared in a three-step synthesis using standard reaction protocols. Chloroethoxyethoxyethanol was oxidized to the corresponding acid using Jones reagent [13] and the chloride converted to azide by displacement with sodium azide in DMF. The azide was reduced to the corresponding amine by hydrogenation (Pd/C).

Cells

Mouse P388 leukemia and Novikoff rat hepatoma

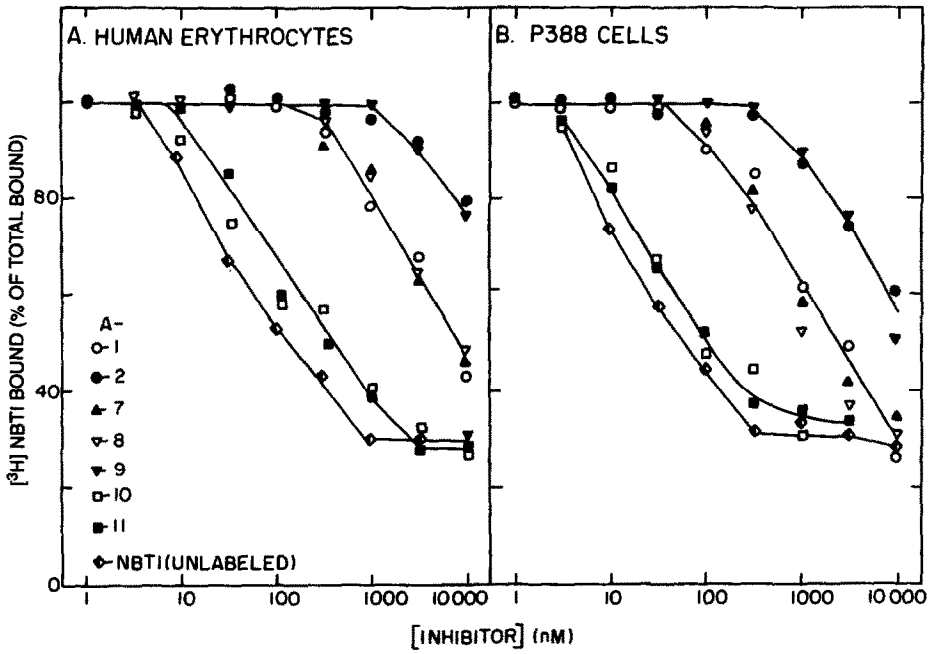

Fig. 2. Effects of NBTI and NBTI derivatives on the equilibrium binding of 1 nM [^{3}H]NBTI by human erythrocytes (A) and mouse P388 leukemia cells (B). The experiments were conducted as described under Materials and Methods. The final cell densities were 6×10^8 human erythrocytes/mL and 2.7×10^7 P388 cells/mL. The control human erythrocytes and P388 cells bound 0.6 to 1 fmol NBTI/10^9 cells and 2.0 to 2.6 fmol/10^8 cells, respectively.

cells were propagated in suspension culture as described previously [14]. Cultures were determined to be free of mycoplasma contamination [15]. For transport experiments, the cells were harvested from late exponential phase cultures and suspended to $2–5 \times 10^7$ cells/mL of basal medium 42 [7].

Outdated human erythrocytes supplemented with a conventional solution of citrate phosphate, dextrose and adenine (CDPA-1) were obtained from the Blood Bank, University of Minnesota Hospitals, stored at 4°, and used within 3 weeks of the expiration date [16]. For experiments, the erythrocytes were washed three times in cold saline buffered with 5 mM Tris–HCl (pH 7.4; Tris-saline) and suspended in the same solution to $2–5 \times 10^9$ cells/mL.

Nucleoside transport measurements

Samples of cell suspensions were supplemented with various concentrations of inhibitors as indicated in appropriate experiments at least 5 min before nucleoside transport was assayed which was sufficient to attain maximum transport inhibition. Time courses of transmembrane equilibration of ^{3}H-labeled nucleosides was measured using a dual syringe apparatus combined with manual sampling for longer time points (12 or 15 time points/time course) as previously described [1, 6, 17]. The procedure involves separating the cells from the medium by rapid centrifugation through an oil layer and analyzing the cell pellet for radioactivity [1, 17]. Radioactivity/cell pellet was corrected for that attributable to trapping in extracellular spaces as estimated with [^{14}C]inulin. Intracellular water space was measured with ^3H_2O [17].

The influx of 10 μM [^{3}H]formycin B (60–90 cpm/pmol) in P388 cells was measured under zero-*trans* conditions at 25°. An integrated rate equation for zero-*trans* influx was fitted to each time course of fomycin B transmembrane equilibration with K_m fixed at 300 μM and the slope at t = 0 was taken as initial velocity (v_{12}^{z}) as described previously [1, 18, 19]. The transmembrane equilibration of 100 μM [^{3}H]uridine by human erythrocytes was measured under equilibrium exchange conditions at 20° [16, 20]. A suspension of erythrocytes was incubated with 100 μM unlabeled uridine at 37° for 1 hr and then the time course of equilibration of [^{3}H]uridine at the same concentration was measured. The velocity of equilibrium exchange (v^{ee}) was estimated by integrated rate analysis with K^{ee} fixed at 400 μM as described previously [16, 20].

Equilibrium binding of NBTI

The equilibrium binding of [^{3}H]NBTI was measured as described previously [6]. In brief, samples of a cell suspension were supplemented with 1 nM [^{3}H]NBTI and where indicated with various concentrations of inhibitors. After 30–60 min of incubation at room temperature, the suspension samples were centrifugally cleared and duplicate samples of the supernatant were analyzed for radioactivity (= free [^{3}H]NBTI). The amount of [^{3}H]NBTI bound was taken as the total amount of [^{3}H]NBTI added minus free [^{3}H]NBTI. In selective

Table 1. Inhibition of [^{3}H]NBTI binding and nucleoside transport by human erythrocytes and P388 mouse leukemia cells by various derivatives of NBTI*

Inhibitor		[^{3}H]NBTI binding		Formycin B transport	Uridine transport
Designation	Substituent groups at	P388 cells IC_{50} (nM)	Human erythrocytes IC_{50} (nM)	P388 cells IC_{50} (nM)	erythrocytes IC_{50} (nM)
A-1	2-	300 (10)†	3,000 (100)	200 (40)	1,000 (125)
A-2	2-	30,000 (1000)	50,000 (1700)	400 (80)	2,000 (250)
A-7	2-	600 (20)	3,000 (100)	6 & 300 (60)	600 (75)
A-8	2-	1,000 (33)	3,000 (100)	300 (60)	600 (75)
A-9	2-	6,000 (200)	30,000 (1000)	200 (40)	2,000 (250)
A-6	5'-	300 (10)	1,000 (33)	12 (2)	60 (8)
A-10	5'-	50 (2)	100 (3)	25 (5)	40 (5)
A-11	5'-	50 (2)	100 (3)	15 (3)	40 (5)
A-3	3'-	1,000 (33)	3,000 (100)	25 (5)	200 (25)
A-4	3'-5'-	3,000 (100)	⩾10,000	1,000 (200)	10,000 (1250)
A-5	2',3',5'	⩾10,000	⩾10,000	800 (190)	>10,000
NBTI	None	30	30	5	8

* Results were derived from the data in Figs. 2 and 3 and similar data from other experiments. All values are approximations based on single or duplicate experiments.

† Values in parentheses indicate the ratio of IC_{50} of *A* derivative/IC_{50} for unlabeled NBTI.

analyses it was shown that maximum inhibition of [^{3}H]NBTI binding by various NBTI derivatives was attained after 30 min of incubation.

Radiolabeled materials

[^{3}H]Formycin B, [5-^{3}H]uridine and [^{3}H]NBTI were purchased from Moravek Biochemicals, Brea, CA.

RESULTS AND DISCUSSION

Figure 2 illustrates representative results of the inhibition by various NBTI derivatives (Fig. 1) of the equilibrium binding of 1 nM [^{3}H]NBTI to human erythrocytes and mouse P388 cells. The IC_{50} values estimated from the data in Fig. 2 and from similar other experiments are summarized in Table 1. All NBTI derivatives with substituents at either the 2-position of the purine moiety or the 3' or 5'-position of the ribose moiety exhibited reduced affinity for the high-affinity binding site, which, however, varied considerably in degree. This conclusion is deduced from the effectiveness of the derivatives in inhibiting the equilibrium binding of [^{3}H]NBTI as compared to that of unlabeled NBTI (IC_{50} = ~30 nM). All IC_{50} values are expressed on the basis of total inhibitor added. Because of the relatively high cell densities employed in these experiments, a considerable proportion of the added inhibitor would be expected to be bound to the cells (specifically and non-specifically) and significantly lower IC_{50} values would therefore be noted if expressed on the basis of free (unbound) inhibitor concentration. In the case of unlabeled NBTI, where this could be calculated, the IC_{50} was about 12 nM when expressed on the basis of free [^{3}H]NBTI for both human erythrocytes and P388 cells. About 30% of the binding of [^{3}H]NBTI to both types of cells was non-saturable and thus non-specific (Fig. 2).

Of interest is that the affinity of NBTI was similarly reduced whether the 2-substituent was hexyldiamine (*A-7*), *N*-acetylhexyldiamine (*A-8*) or [α-*N*-(formyl-furanosulfonic acid) lysine] tetramer (*A-1*). Thus, the size of the substituent group beyond a certain size may not have been a major determinant in reducing binding affinity. Also, slight charge differences seemed to be unimportant. On the other hand, attachment of 6-aminocaproic acid (*A-2*) or aminoethoxyethoxyacetic acid (*A-9*) at the 2-position further reduced the affinity of NBTI for its high-affinity binding site (Table 1). All the 2-derivatives can also be considered derivatives of NBTG, which exhibits about the same affinity for the high-affinity binding site as NBTI [1, 3, 4]. Thus, the attachment of an amino group at the 2-position *per se* does not affect the affinity of the ligand.

Attachment of a succinate group on the ribose moiety reduced the affinity of NBTI in the order 5' < 3' < 3',5' (*A-6*, *-3* and *-4*, respectively; Table 1). The succinate group at the 5'-position had relatively little effect. Similarly, attachment of a (*N*-ethanolamine)-succinate or an {α-*N*-[(5-sulfonate-2-furanyl)methyl]-lysine trimer-alanine} succinate at the 5'-position of NBTI affected relatively little its inhibition of the binding of 1 nM [^{3}H]NBTI (Fig. 2 and Table 1). Thus, 5'-substituents had little effect on the affinity of NBTI to its high-affinity membrane binding site, regardless of the size of the 5'-substituent (at least up to about 1000 kDa; *A-10*). On the other hand, blockage of the 2'- and 3'-OH groups completely abolished binding of NBTI to its binding site as indicated by the failure of *A-5* to inhibit the binding of [^{3}H]NBTI (Table 1).

For assaying the effects of the NBTI derivatives on equilibrative nucleoside transport, we selected two approaches: zero-*trans* formycin B influx in P388 cells and uridine equilibrium exchange in human erythrocytes. The reasons for these approaches are as follows: NBTI-sensitive nucleoside transport represents 70–80% of the total equilibrative

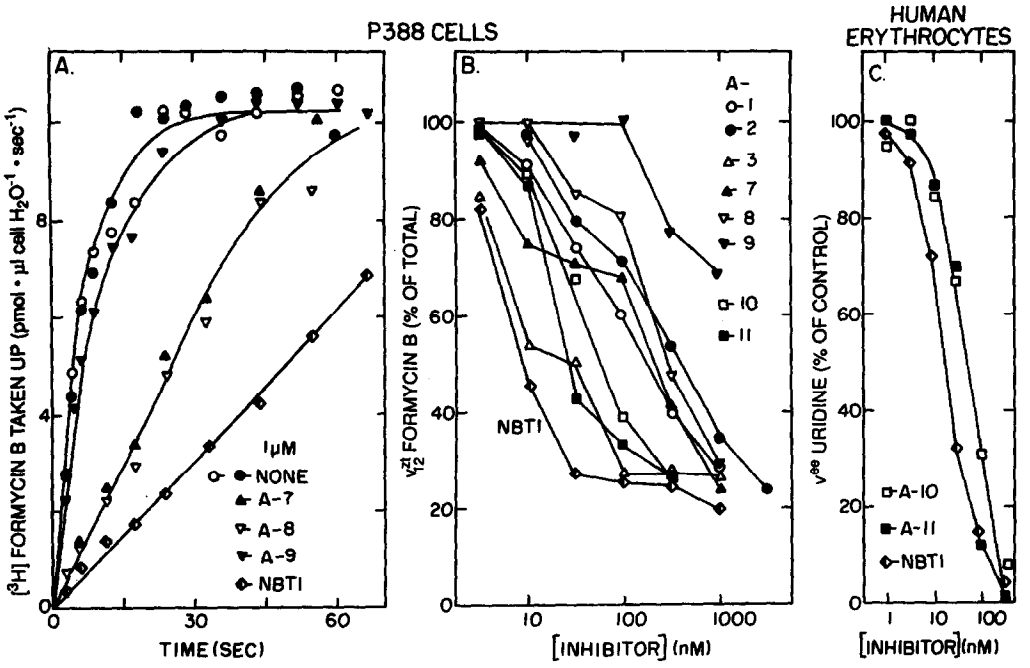

Fig. 3. Effects of NBTI and NBTI derivatives on the zero-*trans* influx of formycin B by P388 cells (A and B) and uridine equilibrium exchange by human erythrocytes (C). (A) Samples of a suspension of 2.7×10^7 P388 cells/mL were supplemented with 3, 10, 30, 100, 300 and 1000 nM NBTI or of the indicated NBTI derivatives and then the transmembrane equilibration of 10 μM [^{3}H]formycin B (8.3 cpm/pmol) was measured as described under Materials and Methods. Only the progress curves for the untreated cells and 1 μM concentrations of the inhibitors are presented. Initial transport velocities (v_{12}^{z}) were estimated by integrated rate analysis with the Michaelis-Menten constant (K_{12}^{z}) fixed at 300 μM [18, 19]. The calculated v_{12}^{z} values are plotted along with values obtained from another comparable experiment as percent of v_{12}^{z} of untreated control cells (1 pmol · μL cell H_2O^{-1} · sec^{-1}) as a function of the inhibitor concentration in frame B. (C) A suspension of 1.2×10^9 erythrocytes/mL was equilibrated with 100 μM unlabeled uridine. Then samples of the suspension were supplemented with 1, 3, 10, 30, 100 and 300 nM NBTI, *A-10* or *A-11* and the time courses of inward equilibrium exchange of 100 μM [^{3}H]uridine (2 cpm/pmol) were measured. Initial exchange velocities (v^{ee}) were estimated by integrated rate analysis with the Michaelis–Menten constant (K^{ee}) fixed at 400 μM [21]. The calculated v^{ee} values were plotted as percent of v^{ee} of untreated control cells (15 pmol · μL cell H_2O^{-1} · sec^{-1}) as a function of inhibitor concentration.

nucleoside transport activity of P388 cells; the remainder represents NBTI-resistant transport [6]. Thus, if a chemical modification of NBTI alters its specificity for NBTI-sensitive transport this should become apparent using P388 cells. Formycin B, a C-analog of inosine, was selected as substrate because it is an efficient substrate for both the NBTI-sensitive and NBTI-resistant nucleoside transporters, but is only very poorly phosphorylated by the cells and resistant to enzymatic phosphorolysis [18, 19]. Thus, it is an ideal substrate for measuring nucleoside transport unimpeded by metabolic conversions [18, 19]. The same is true for uridine transport by human erythrocytes, since these cells lack uridine kinase and uridine phosphorylase activities, whereas uridine is a natural and efficient substrate for the nucleoside transporter of these cells [20, 21]. Furthermore, human erythrocytes were selected because they express only NBTI-sensitive equilibrative nucleoside transport and because their transporter differs kinetically from that found in other types of mammalian cells including P388 cells [1, 20, 21]. The carrier of the latter exhibits directional symmetry and equal mobility whether empty or substrate loaded. In contast, the carrier of human erythrocytes moves more rapidly when substrate-loaded than when empty [1, 20, 21].

The reduction in the ability of the NBTI derivatives to inhibit [^{3}H]NBTI binding to its high-affinity binding site generally correlated well with the reduction in their ability to inhibit the transport of formycin B by P388 cells or of uridine by human erythrocytes (Table 1). The exceptions were *A-2*, *A-9*, *A-6* and *A-3* (see later). Examples of representative raw data illustrating the inhibition of formycin B influx by P388 cells are shown in Fig. 3, A and B. Initial transport velocities (v_{12}^{z}) were estimated by integrated rate analysis of the time courses of transmembrane equilibration of formycin B (Fig. 3A) and are presented as a function of inhibitor concentration in Fig. 3B. The IC_{50} values estimated from the data in Fig. 3B and from other similar experiments are summarized in Table 1. Comparison of the values in Table 1 shows that *A-2*,

A-9, *A-6* and *A-3* inhibited nucleoside transport by both P388 cells and human erythrocytes more effectively than the binding of [^{3}H]NBTI to these cells. The reason for this difference is unknown. However, both *A-2* and *A-9* possess terminal acetates on the 2-substituent groups and *A-3* and *A-6* possess succinate substituents at the 3'- and 5'-positions, respectively, which may have reduced the effectiveness of these derivatives to interfere with the binding of nucleosides to the carrier substrate binding site less than their affinity for the lipophilic domain of the NBTI binding site. It is of interest, however, that dual attachment of succinates at both the 3'- and 5'-positions of NBTI (*A-4*) almost completely abolished its affinity for the high-affinity binding site and its ability to inhibit nucleoside transport. To what extent these differences are related to alterations of the lipophilicity of NBTI by the substituent groups is not clear at present.

Another unusual result was that the inhibition of formycin B influx by P388 cells by *A-7* (NBTI-2-hexyldiamine) seemed to be biphasic (Fig. 3B and Table 1). This was observed in a second independent experiment, but the reason for this observation is also not known. It could be due to the presence of small amounts of unlabeled NBTI (or NBTG) in the inhibitor preparation, but this seems unlikely since the inhibition of uridine exchange in human erythrocytes and of [^{3}H]NBTI binding by *A-7* was not biphasic (Fig. 2). The inhibition of formycin B transport by *A-3* and *A-8* might also be biphasic (Fig. 3B), but the breaks in the inhibition curves were slight and not observed for *A-3* in a second experiment.

None of the derivatives at a concentration of 1 μM had any effect on NBTI-resistant formycin B transport by P388 cells, which represented 25–30% of the total transport activity of the cells (Fig. 3B). Formycin B transport by Novikoff rat hepatoma cells, which express only NBTI-resistant nucleoside transport [1], was also not affected by any of the NBTI derivatives at a concentration of 1 μM (data not shown). In contrast, all inhibitory derivatives of NBTI, like NBTI itself, completely inhibited the equilibrium exchange of uridine in human erythrocytes. This finding is exemplified by the data in Fig. 3C.

Also of interest is that all substituents reduced the affinity of NBTI for the high-affinity binding site of human erythrocytes to a greater extent than that of P388 cells (Table 1). It is unclear whether this differential effect was mediated at the level of the hydrophobic domain of the high-affinity binding site or the nucleoside binding site of the carrier that is part of this site. We suspect that the latter is the case since this difference was reflected by a similar difference in the inhibition of formycin B transport in P388 cells and uridine transport in human erythrocytes (Table 1) and because of the known differences in the kinetic properties of the nucleoside transporters of the two types of cells, whereas the K_d of NBTI binding is about the same for these cell types [1, 6].

In summary, substituents at the 5'-position of NBTI, regardless of size, had relatively little effect on its binding to high-affinity membrane NBTI binding sites or its inhibition of NBTI-sensitive nucleoside transport. Substitutents at the 2-position greatly reduced NBTI binding and transport inhibition but the effect was not simply related to the size of the substituent group. Substituents at the 3'-position also reduced NBTI binding but most striking was the effect of blocking of the 2',3'-OH groups of NBTI which almost completely abolished its binding to the high-affinity binding site as well as its inhibition of nucleoside transport (Table 1). Thus, the interaction of the 2',3'-OH groups with the nucleoside carrier plays an important role in the binding of NBTI to its high-affinity binding site. We have demonstrated previously that the 2'- and 3'-OH groups play an important role in nucleoside substrate recognition by the carrier [1, 21–23]. 2'-Deoxynucleosides are transported as efficiently as ribonucleosides [1, 21], but 3'-deoxyadenosine (cordycepin) is less efficiently transported [21] and 2'-3'-deoxynucleosides are very poorly transported (2',3'-dideoxycytidine) or not at all (2',3'-dideoxyadenosine and azidothymidine) [21–24].

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REFERENCES

1. Plagemann PGW, Wohlhueter RM and Woffendin C, Nucleoside and nucleobase transport in animal cells. *Biochim Biophys Acta* **947**: 405–443, 1988.
2. Wohlhueter RM, Brown WE and Plagemann PGW, Kinetic and thermodynamic studies on nitrobenzylthioinosine binding to the nucleoside transporter of Chinese hamster ovary cells. *Biochim Biophys Acta* **731**: 168–176, 1983.
3. Paul B, Chen MF and Paterson ARP, Inhibitors of nucleoside transport. A structure–activity study using human erythrocytes. *J Med Chem* **18**: 968–973, 1975.
4. Paterson ARP, Jakobs ES, Harley ER, Fu NW, Robins MS and Cass CE, Inhibition of nucleoside transport. In: *International Symposium on Adenosine* (Eds. Berne RM, Rall TW and Rubio R), pp. 203–217. Martinus Nijhoff, The Hague, 1982.
5. Belt JA, Heterogeneity of nucleoside transport in mammalian cells. Two types of transport activity in L1210 and other cultured neoplastic cells. *Mol Pharmacol* **24**: 479–484, 1983.
6. Plagemann PGW and Wohlhueter RM, Nucleoside transport in cultured mammalian cells. Multiple forms with different sensitivity to inhibition by nitrobenzylthioinosine or hypoxanthine. *Biochim Biophys Acta* **773**: 39–52, 1984.
7. Plagemann P and Aran JM, Characterization of Na^+-dependent, active nucleoside transport in rat and mouse peritoneal macrophages, a mouse macrophage cell line and normal rat kidney cells. *Biochim Biophys Acta* **1028**: 289–298, 1990.
8. Gerster JF and Robins RK, Purine nucleosides. X. The synthesis of certain naturally occurring 2-substituted amino-9-β-D-ribofuranosylpurin-6(1H)-ones (N^2-substituted guanosines). *J Am Chem Soc* **87**: 3752–3759, 1965.
9. Kawana M, Srivastava PC and Robins RK, Nucleoside peptides. Synthesis of certain *N*-(9-β-D-ribofuranosylpurin-2-yl)-L-amino acids. *J Carbohyd Nucleosides Nucleotides* **8**: 131–144, 1981.
10. Chladec S and Smirt J, Oligonucleotidic compounds.

V. 2′-3′-*O*-Alkylidene derivatives of ribonucleosides. *Collect Czech Chem Commun* **28**: 1301–1308, 1963.
11. Matsuda A, Shinozaki M, Suzuki M, Watanabe K and Miyasaka T, A convenient method for selective acylation of guanine nucleosides. *Synthesis* 385–386, 1986.
12. Evans ME, Parrish FW and Long L Jr, Acetal exchange reactions. *Carbohydr Res* **3**: 453–462, 1967.
13. Baumgarten HE (Ed.), *Organic Syntheses*, Vol. 5, p. 310. John Wiley, New York, 1973.
14. Plagemann PGW and Wohlhueter RM, The hypoxanthine transporter of Novikoff rat hepatoma cells exhibits directional symmetry and equal mobility when empty or substrate-loaded. *Biochim Biophys Acta* **688**: 505–514, 1982.
15. Plagemann PGW, Mycoplasma contamination greatly enhances the apparent transport and concentrative accumulation of formycin B by mammalian cell culture. *Biochim Biophys Acta* **1064**: 162–164, 1991.
16. Plagemann PGW and Wohlhueter RM, Kinetics of nucleoside transport in human erthrocytes. Alterations during blood preservation. *Biochim Biophys Acta* **778**: 176–184, 1984.
17. Wohlhueter RM and Plagemann PGW, Measurement of transport versus metabolism in cultured cells. *Methods Enzymol* **173**: 714–733, 1989.
18. Plagemann PGW and Woffendin C, Use of formycin B as a general substrate for measuring facilitated nucleoside transport in mammalian cells. *Biochim Biophys Acta* **1010**: 7–15, 1989.
19. Plagemann PGW and Aran JM, Na^+-dependent, active nucleoside transport in mouse spleen lymphocytes, leukemia cells, fibroblasts and macrophages, but not in equivalent human or pig cells; dipyridamole enhances nucleoside salvage by cells with both active and facilitated transport. *Biochim Biophys Acta* **1025**: 32–42, 1990.
20. Plagemann PGW, Wohlhueter RM and Erbe J, Nucleoside transport in human erythrocytes. A simple carrier with directional symmetry and differential mobility of loaded and empty carrier. *J Biol Chem* **257**: 12069–12074, 1982.
21. Plagemann PGW, Aran JM, Wohlhueter RM and Woffendin C, Mobility of nucleoside transporter of human erythrocytes differs greatly when loaded with different nucleosides. *Biochim Biophys Acta* **1022**: 103–109, 1990.
22. Plagemann PGW and Woffendin C, Dideoxycytidine permeation and salvage by mouse leukemia cells and human erythrocytes. *Biochem Pharmacol* **38**: 3469–3475, 1989.
23. Plagemann PGW and Woffendin C, Permeation and salvage of dideoxyadenosine in mammalian cells. *Mol Pharmacol* **36**: 185–192, 1989.
24. Zimmerman TP, Mahony WB and Prus KL, 3′-Azido-3′-deoxythymidine. An unusual nucleoside analogue that permeates the membrane of human erythrocytes and lymphocytes by nonfacilitated diffusion. *J Biol Chem* **262**: 5748–5754, 1987.