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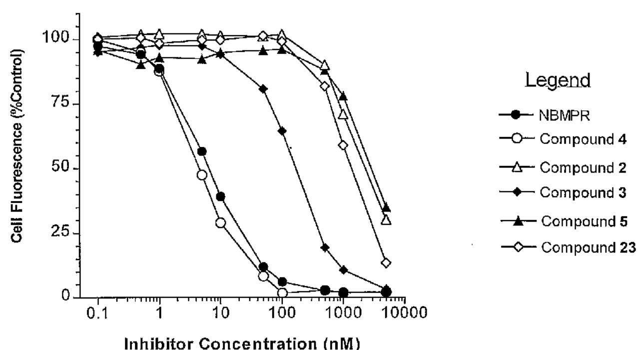
(54) Title: NOVEL NUCLEOSIDE TRANSPORT INHIBITORS



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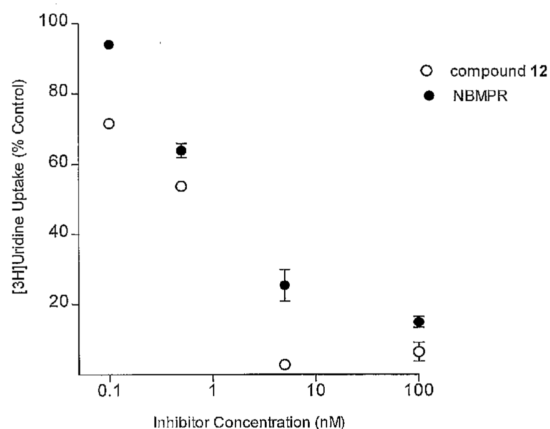

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(57) Abstract: Compounds or compositions that are inhibitors and/or ligands of nucleoside transporters; and methods of treating cancer, heart disease and stroke, as well as AIDS and other infectious diseases

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

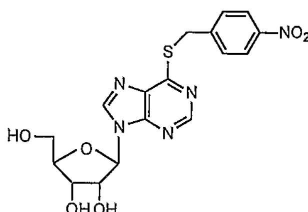
NOVEL NUCLEOSIDE TRANSPORT INHIBITORS

Field of the Invention

5 The present invention relates to the field of compounds that bind to the cellular nucleoside uptake proteins termed nucleoside transporters. The compounds help prevent *in vivo* metabolic inactivation and production of toxic metabolites that is associated with the available related nucleoside transport inhibitors. These compounds are thus novel therapeutic agents in cancer, heart disease and stroke, as 10 well as AIDS and other infectious diseases.

Background of the Invention

Mammalian cells take up, and release physiological nucleosides and many of their synthetic analogs primarily by means of specific integral plasma membrane 15 glycoproteins known as nucleoside transporters (Paterson and Cass, 1986; Plagemann *et al.*, 1988; Gati and Paterson, 1989; Paterson *et al.*, 1991; Cass, 1995; Thorn and Jarvis, 1996). Nucleoside transporters have been classified into two categories: (i) equilibrative (facilitated diffusion) and (ii) concentrative (secondary active) sodium-dependent. Two equilibrative transporters with similar broad substrate specificities 20 have been identified and designated as the *es* (equilibrative sensitive) and *ei* (equilibrative insensitive) transporters, on the basis of their sensitivity or insensitivity to inhibition by nitrobenzylthioinosine (NBMPR, **1**), respectively. Six sodium ion-coupled (concentrative) transporters designated *cif*/N1, *cit*/N2, *cib*/N3, *cit*/N4, *cs*/N5 and *csg*/N6 have also been identified in mammalian tissues (Cass, 1995, Young *et al.*, 25 2000). However, sodium-dependent nucleoside transport is only a minor component of mammalian tissues and plays a role mainly in secretory tissues. The *es* transporter is by far the major nucleoside transporter of most mammalian tissues, especially heart tissue (Williams, 1996; Hoehner *et al.*, 1996; Abd-Elfattah *et al.*, 1998a), and is highly sensitive to inhibition by NBMPR, and related purine 6-position (nitrobenzyl) 30 nucleosides (Paul *et al.*, 1975; Robins *et al.*, 1994; Paterson *et al.*, 1983), and has a high affinity for NBMPR (K_d in the 0.1-1.0 nM range).



1, NBMPR

5

In addition to their role as precursors for salvage synthesis of nucleotides used for DNA and RNA synthesis, physiological nucleosides are also involved in signal transduction and metabolic pathways. Adenosine is especially involved in protecting tissues from ischemic and inflammatory damage (Engler, 1987; Ohta and Sitkovsky, 10 2001). Adenosine's tissue protective effects can be harnessed for the treatment of ischemic heart disease and stroke, as wells as other ischemic conditions and the preservation of donor hearts and kidneys (reviewed in Buolamwini, 1997). Agents that potentiate endogenous adenosine's protective effects are therefore being pursued. One attractive potential strategy for *in vivo* adenosine potentiation is adenosine 15 transport blockade (Van Belle, 1993a, 1993b). Adenosine is automatically released endogenously in myocardial infarction or stroke, as a "retaliatory" metabolite (Newby, 1984) through ATP catabolism to protect against ischemic tissue damage through its interaction with cell surface G-protein coupled adenosine receptors (Ver Donck, 1994; Linden, 2001). However, adenosine's protective effects are quickly lost 20 mainly by cellular uptake through nucleoside transporters (Van Belle, 1993a), which contribute to its depletion from the extracellular milieu. The adenosine potentiation effects of nucleoside transporter inhibitors, which help maintain extracellular adenosine concentrations, have been demonstrated in heart and brain ischemia models (Van Belle, 1993a, 1993b; Abd Elfattah and Wechsler, 1994; Abd-Elfattah *et al.*, 25 1998b; Rudolphi *et al.*, 1992; Parkinson *et al.*, 2000; Zhang *et al.*, 2002). The benefit of using NT inhibitors rather than adenosine receptor agonists is that the effects of NT inhibitors, unlike adenosine receptor agonists are localized (Van Belle, 1993b) to tissues where adenosine is released locally in an ischemic episode, making this approach event- and site-specific. Adenosine receptor agonists on the other hand will

trigger adenosine receptors all over the body leading to unwanted side effects, a major limitation in the drug development of adenosine receptor agonists (Erion, 1993). Other less attractive adenosine enhancement strategies include the use of adenosine deaminase (ADA) inhibitors such as deoxycoformycin (Phillis and O'Regan, 1996). The disadvantage in using ADA inhibitors is that they are prone to cause severe combined immunodeficiency (SCID), a condition that is observed in a genetic deficiency in the production of the enzyme (Strachan and Read, 1996).

The combination of NT inhibitors with *de novo* synthesis inhibitor antimetabolite chemotherapy in cancer and infectious diseases is also of considerable interest (Buolamwini, 1997). Thus, dipyridamole has been shown to enhance the antitumor effects of methotrexate (Cabral *et al.*, 1984) and 5-fluorouracil (Grem and Fischer, 1985). The experimental and clinical studies relating to the modulation of 5-fluorouracil by nucleoside transport inhibitors have been reviewed (Tew *et al.*, 1993). The continued interest in this therapeutic approach is shown by a recent prospective randomized clinical trial that tested the efficacy of a combination of orally administered dipyridamole with leucovorin and 5-FU in advanced colorectal cancer (Kohne *et al.*, 1995).

The available NT inhibitors, however, lack the requisite pharmacological profiles are toxic, mutagenic, ineffective *in vivo*, or nonspecific (reviewed in Buolamwini, 1997), and therefore the above-mentioned therapeutic strategies will benefit tremendously from the discovery of better nucleoside transport inhibitors.

In this regard, the compounds that are the subject of this disclosure are invented to accomplish two goals: (i) to provide conformationally restrained analogs of NBMPR to be used as probes of the bioactive conformation of NBMPR for better rational nucleoside transporter inhibitor design and (ii) to overcome a major disadvantage of NBMPR, which has to do with the lability of the S^6-nitrobenzyl substituent, which is easily lost *in vivo* to drastically reduce NT inhibitory activity (up to a 1000 fold). This lability has actually allowed NBMPR to be used as an effective photolabeling reagent for the *es* nucleoside transporter (Young *et al.*, 1983; Boumah *et al.*, 1992). Incorporating the high potency conferring nitrobenzyl group of NBMPR into a nitrotetrahydroisoquinoline system prevents it from being easily cleaved. At the same time this molecular modification restrains the conformational flexibility at

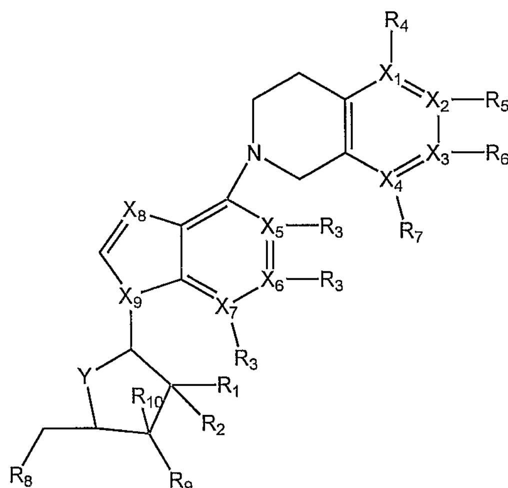
the purine 6-position substituents and helps to better map the orientation of the nitrobenzyl group when bound to the *es* transporter.

Novel compounds are synthesized and their binding affinity at the *es* nucleoside transporter evaluated by a flow cytometric assay (Buolamwini *et al.*, 5 1994). Their inhibition of nucleoside uptake has also been demonstrated, as well as their ability to enhance the recovery of isolated heart preparation from global ischemia.

10 Summary of the Invention

The present invention is directed to compounds or compositions that are inhibitors and/or ligands of nucleoside transporters for research and therapeutic uses. These compounds include four isomeric aromatic nitro-1,2,3,4-tetrahydroisoquinolinylpurine ribosides and appropriate derivatives thereof.

15 The present invention includes, but is not limited to, the following compounds, as well as analogs thereof:



20 FORMULA I

wherein:

X_1-X_9 are each independently C, N, provided that where X is N, the R substituent is absent;

Y is O, S, NH, CH_2;

R_1 is H, OH, NH_2, N_3, halogen;

R_2 is H, OH, NH_2, N_3, halogen;

R_3 is H, NH_2, substituted amino, halogen;

5 R_4 is H, OH, NO_2, CN, N_3, NCS, halogen;

R_5 is H, OH, NO_2, CN, N_3, NCS, halogen, halogen-CH_2COCH_3;

R_6 is H, OH, NO_2, CN, N_3, NCS, halogen, halogen-CH_2COCH_3;

R_7 is H, OH, NO_2, CN, N_3, NCS, halogen;

R_9 is H, OH, NH_2, N_3, halogen;

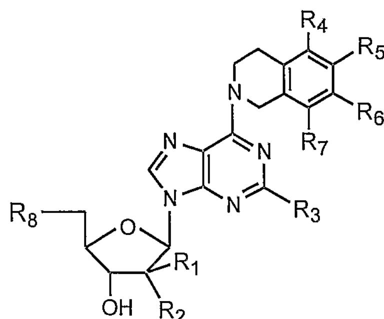
10 R_8 is H, OH, OCH_3, NO_2, CN, N_3, NCS, halogen, A-Reporter (where A is O, S, NH, and the Reporter is a tethered reporter group;

R_{10} is H, OH, NH_2, N_3, halogen;

provided that when X_1-X_4, X_6, are all C, X_5, X_7, X_8, X_9 are all N, and Y is O, substituents R_4, R_5, R_6, R_7, cannot all be H at the same time if R_1 is H, R_2 is OH, R_3 is H

15 and R_8 is OH.

Other embodiments of the present invention include the following compounds and analogs thereof:



FORMULA II

R_1 = H, OH, Halogen
R_2 = H, OH, Halogen
R_3 = H, NH_2 or substituted amino, Halogen
R_4 = H, OH, NO_2, CN, N_3, NCS, Halogen
R_5 = H, OH, NO_2, X, CN, N_3, NCS, X-CH_2COCH_3 (where X is Cl, Br or I)
R_6 = H, OH, NO_2, X, CN, N_3, NCS, X-CH_2COCH_3 (where X is Cl, Br or I)
R_7 = H, NO_2, OH, CN, N_3, NCS, Halogen
R_8 = H, OH, OCH_3, NO_2, CN, N_3, NCS, Halogen, A-Reporter (where A is O, S, NH and the Reporter is a tethered reporter group such as a fluorophore (fluorescein, texas red, Cy3, Cy5, Bodipy, phycoerythrin or other fluorophore), biotin, or radioactive group

5 provided that substituents R_4, R_5, R_6, R_7, cannot all be H at the same time if R_1 is H, R_2 is OH, R_3 is H and R_8 is OH.

The present invention also comprises pharmaceutical formulations of the above compounds, including pharmaceutically acceptable acid addition salts, esters, or prodrugs thereof.

10 The present invention also relates to methods of treating cancer, heart disease, strokes, AIDS, and infectious diseases comprising administering a compound or composition of the present invention to a subject in need thereof.

15 Brief Description of the Drawing

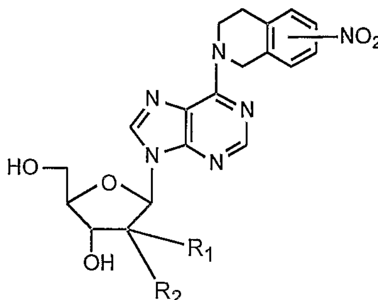
Figure 1 is a graph showing the equilibrium displacement of SAENTA-fluorescein ligand by of new tetrahydroisoquinoline constrained NBMPR analogs in K562 cells. Cells are incubated with 25 nM SAENTA fluorescein in the presence of inhibitor for 45 minutes at room temperature and analyzed by flow cytometry.

Figure 2 is a graph showing inhibition of [^{3}H]uridine uptake by NBMPR (•) and a novel tetrahydroisoquinoline analogue compound 12 (○) in JB6 cells. Cells in logarithmic phase of growth are incubated with 1 μM [^{3}H]uridine and 10-second uptake measurements were performed as described in Paterson *et al.* (1983).
5 The data points are mean ± std for triplicate samples.

Detailed Description of the Invention

10 As stated above, the present invention relates to nucleoside transport inhibitors.

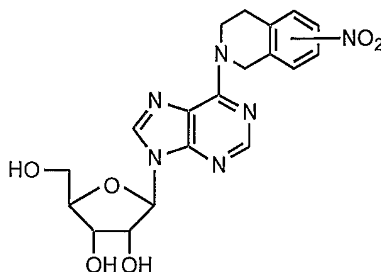
In other embodiments of the present invention, the present invention is directed to the following compounds and analogs thereof:



15

wherein R_1 and R_2 are described above.

In other embodiments, the present invention is directed to the following
20 compounds and analogs thereof:



The present invention also comprises pharmaceutical compositions/formulations of all the compounds of the present invention, including pharmaceutically acceptable acid addition salts, esters, or prodrugs thereof. Compounds of the invention and their physiologically acceptable salts may be administered by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). The preferred route of administration may vary with for example the condition of the recipient. While it is possible for the active ingredients to be administered alone it is preferably to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

As stated above, the present invention also relates to methods of treating cancer, heart disease, strokes, AIDS-related diseases, and infectious diseases comprising administering a compound or composition of the present invention to a subject in need thereof.

Examples of forms of cancers particularly suitable for treatment with the compounds of the present invention are malignant melanoma, cervical cancer, breast cancer, colorectal cancer, color cancer, cancer of the head and neck, gastric cancer, renal cancer, laryngeal cancer, rectal cancer, non-Hodgkins lymphoma and leukemias.

The compounds of the present invention may be used to treat and inhibit heart disease and strokes, including ischemic heart disease and stroke, as well as ischemic conditions of kidney and liver, thereby allowing the preservation of these organs for transplant purposes.

According to a further aspect of the present invention provides for the use of the compounds of the present invention for the treatment and/or prophylaxis of acute

and chronic infectious diseases. Examples of acute viral infections are: Herpes viruses, influenza viruses, parainfluenza viruses, adenoviruses, coxsakie viruses, picorna viruses, rotaviruses, heptatis (A, B, C, D) virus, mumps virus, rubella virus, measles virus, pox viruses, respiratory syncytial viruses, papilloma viruses, and
5 enteroviruses, arenavirus, rhinoviruses, poliovirus, Newcastle disease virus, rabies virus, arboviruses. Examples of chronic viral infections are: persistent herpes virus infections, Epstein Barr virus infection, persistent rubella infections, papovirus infections, hepatitis virus infections and human immunodeficiency virus infection.

The compounds of the invention may be used in the treatment or prophylaxis
10 of retroviral, gram-negative bacterial, fungal and protozoal infections and in the manufacture of a medicament for the treatment or prophylaxis of a retroviral, gram-negative bacterial, fungal and protozoal infection. Examples of bacteria include: Escherichia coli, Salmonella dublin, Salmonella typhosa, Salmonella typhimurium, Shigella flexneri, Citrobacter freundii, Klebsiella pneumoniae, Vibrio cholerae, Vibrio
15 anquillarum, Enterobacter aerogenes, Pasteurella multocida, Haemophilus influenzae, Yersinia enterocolitica, Pasteurella haemolytica, Proteus mirabilis and Proteus vulgaris, the causative organisms of such ailments as travellers' diarrhoea, urinary tract infections, shigellosis, typhoid fever and cholera in humans, as well as animal diseases such as calf neonatal enteritis, pig post-weaning enteritis and chicken
20 colisepticaemia. Examples of fungi include Candida, Aspergilus and pneumocistis carinii. Examples of protozoa include Plasmodium, Giadia, and Toxoplasma.

As stated above, the compounds of the present invention may be, or may be part of an effective treatment or preventative against AIDS-opportunistic diseases, including the following viruses: human T-cell lymphotropic viruses (HTLV),
25 especially HTLV-I, HTLV-II and HTLV-III (HIV); feline leukaemia virus, equine infectious anaemia virus, caprine arthritis virus and other lentiviruses, as well as other human viruses such as hepatitis B virus, Epstein-Barr virus (EBV) and the causative agent of multiple sclerosis (MS).

30 The activity of the compounds according to the invention against such a wide range of bacterial, fungal, protozoal and viral infections is clearly of great advantage in medicine, and the novel mode of action allows the use of these compounds either

alone or in combination therapy with know agents to reduce the chance of resistance developing. The compounds of the present invention may be used in the combination therapy with known medicaments, as the compounds of US 5,064,946, incorporated herein by reference.

5 The amounts required of the active ingredient will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician or veterinarian.

10

Examples

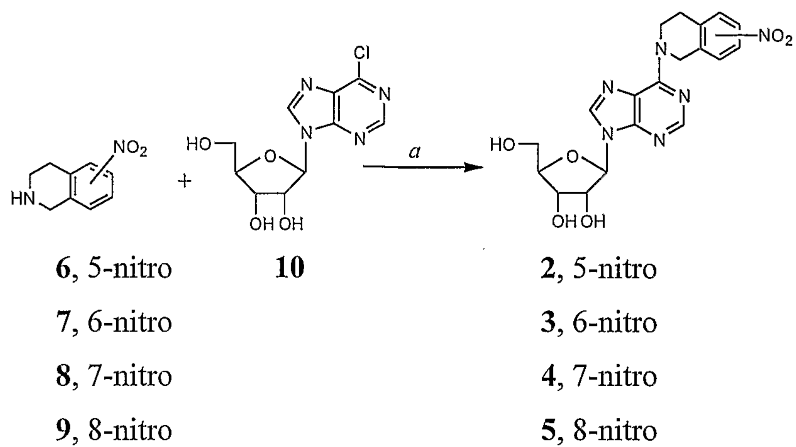
The following Examples show how to make and use the present invention. The specific compounds set forth herein are for exemplary purposes only and are not intended to limit the scope of the present invention in any way.

15

Example 1

This example shows how to make example compounds of the present invention. More specifically, this example demonstrates how to make four aromatic nitro isomers of 6-position nitro-1,2,3,4-tetrahydoisoquinolinylpurine ribosides NBMPR analogues (compounds **2-5**). Several literature methods are employed to
20 synthesize the required nitro-1,2,3,4-tetrahydoisoquinoline (compounds **6-9**), which are subsequently reacted with the commercially available 6-chloropurine riboside (**10**) according to Scheme 1 to obtain the target compounds. The commercially available 5-nitroisoquinoline (**11**) is reduced using sodium borohydride in ethanol to afford the 5-tetrahydroisoquinoline (**6**) intermediate in good yield (about 90 %). The 7-
25 nitrotetrahydroisoquinoline intermediate (**8**) is prepared in about 50 % yield by nitration of the commercially available 1,2,3,4-tetrahydroisoquinoline using KNO_3 in concentrated sulfuric acid according to the method of Ajao *et al.* (1985) (Scheme 3). The 6- and 8-nitro-1,2,3,4-tetrahydroisoquinoline intermediates **7** and **9**, are prepared following reaction Scheme 4 according to the report by Tercel *et al.* (1996) as
30 follows.

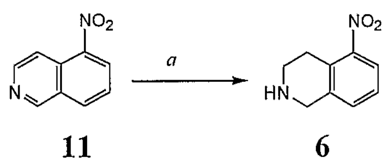
Scheme 1



6, 5-nitro	**10**	**2**, 5-nitro
7, 6-nitro		**3**, 6-nitro
8, 7-nitro		**4**, 7-nitro
9, 8-nitro		**5**, 8-nitro

a, $CaCO_3$/Ethanol

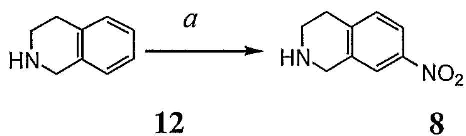
Scheme 2



11 **6**

a, $NaBH_4$/EtOH, 0 °C

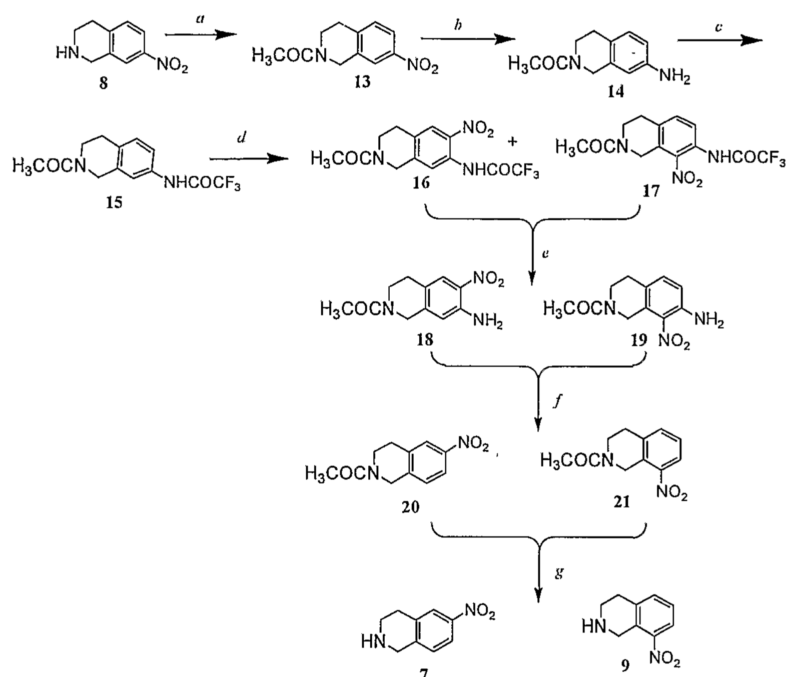
Scheme 3



12 **8**

a, KNO_3/Conc. H_2SO_4, 5 °C

Scheme 4



Reagents and conditions:

a, $(Ac)_2O/Et_3N$, CH_2Cl_2; *b*, $NH_2NH_2.xH_2O/FeCl_3.6H_2O$/Activated carbon/MeOH/reflux; c, TFA/CH_2Cl_2; *d*, KNO_3/conc. H_2SO_4; *e*, HCl/MeOH, reflux; *f*, $NaNO_2$/HCl, H_3PO_2; g, conc.HCl/MeoH, reflux

The 7-nitro-1,2,3,4-tetrahydroisoquinoline intermediate (**8**) used to synthesize compound **4** (see Scheme 2) is protected by acetylation to give compound **13**. The NO_2 group of **13** is reduced to an NH_2 group to obtain compound **14**. The NH_2 group of **14** is subsequently protected and the resulting compound **15** is nitrated to afford the nitro compounds **16** and **17**. Compounds **16** and **17** are deprotected to give free NH_2 substituted compounds **18** and **19**, which are subjected to diazotization using $NaNO_2$ and HCl in the presence of H_3PO_3 to replace the NH_2 by H, to obtain compounds **20** and **21**. Compounds **21** and **22** are deprotected to obtain the required intermediates **7** and **9**. Compound **22**, which is the analog of compounds **2-5** without the nitro substituents, is also synthesized and tested for comparison. Its synthesis is achieved by reacting compound **8** with compound **10** according to the reaction in Scheme 1.

The results show that these compounds are able to bind to the *es* transporter in K562 cells with different affinities. Compound **3** is the most tightly bound to the transporter, among the new compounds, being bound as tightly as the prototype compound, NBMPR (see Figure 1). Compound **4** followed it, which also has IC_{50} value in the low nanomolar range see Figure 1). Compounds **2** and **5** are much less potent inhibitors of SAENTA-fluor binding to the *es* transporter compared to compounds **3** and **4**.

Example 2 - Experimental

Chemistry. Thin-layer chromatography (TLC) is conducted on silica gel F_{254} plates (Analtech). Compounds are visualized by UV light or 5 % H_2SO_4 in EtOH spraying reagent. ^1H, ^{13}C spectra are recorded on Bruker ARX (300 MHz) instruments, using $CDCl_3$, CD_3OD, $(CD_3)_2SO$ or CD_3COCD_3 as solvents and tetramethylsilane (TMS) as internal standard. Flash column chromatography is performed on Fisher silica gel (170-400 mesh). Melting points are determined using a Fisher-Johns Melting Point Apparatus and are reported uncorrected. Mass spectra are obtained on a Bruker-HP Esquire-LC mass spectrometer, and IR spectra in KBr with a Perkin Elmer (System 2000 FT-IR) spectrometer. All solvents and reagents are bought from Aldrich and used without further purification.

Example 3 - General Method for Preparation of Compounds **2**, **3**, **4**, **5**, **22**

A mixture of 6-chloropurine riboside (**10**, 100 mg, 0.35 mmol), (mono-NO_2)-1,2,3,4-tetrahydroisoquinoline (0.88 mmol) and calcium carbonate (70 mg, 0.70mmole) in EtOH (5ml) is stirred under refluxing for 15 hours. The reaction mixture is filtered and the filtrate is removed by rotavapor. The residue is purified by chromatography followed by recrystalization in MeOH.

6-{[mono- (5, 6, 7, or 8)-NO_2-] 1,2,3,4-tetrahydroisoquino-2-yl}purine riboside (Compounds 2, 3, 4, 5, 22). Compound 2: yield 65%; mp 159-160^{0}C; IR: 3438, 1604, 1528, 1352 cm^{-1}; ^1H NMR (300MHz,$CDCl_3$) δ 8.15 (1H, s, H-2), 7.86

(1H, d, H-6″), 7.84 (1H, s, H-8), 7.49 (1H, d, H-8″), 7.36 (1H, t, H-7″), 6.53 (1H, br d, OH-2′), 5.81 (1H, d, H-1′), 5.36 (1H, br s, OH-3′), 5.07 (1H, q, H-2′), 4.53-4.45 (3H, br m, OH-5′, H-3′, 1″A), 4.35 (1H, s, H-1″B), 3.94 (1H, d, H-4′), 3.76 (2H, br m, H-5′), 3.28 (2H, t, H3″ or H4″). Anal. Calcd. For $C_{19}H_{20}N_6O_6$ (428.405): C, 53.27%; H,
5 4.71%; N, 19.62%. Found: C, 53.14%; H, 4.76%; N, 19.32%. **Compound 3:** yield 51%; mp 127.5-129.5^{0}C; MS (ESI) m/z 451 $(M+Na)^+$; IR: 3437, 1593, 1523, 1350 cm^{-1}; ^{1}H NMR (300MHz,CD_3OD) δ 8.30 (1H, s, H-2), 8.29 (1H, s, H-8), 8.10 (2H, m, H-5″, 7″), 7.50 (1H, d, H-8″), 5.98 (1H, d, H-1′), 5.50 (2H, br s, OH-2′, 3′), 4.74 (1H,t, OH-5′), 4.58 (3H, br s, H-2′, 3′, 1″A), 4.32 (1H, q, H-4′), 4.17 (1H,d, H-1″B)
10 3.89 (1H, q, H-5′A), 3.74 (1H, q, H-5′B), 3.15 (2H, t, H3″ or H4″). Anal. Calcd. For $C_{19}H_{20}N_6O_6.H_2O$ (446.420): C, 51.12%; H, 4.97%; N, 18.82%. Found: C, 51.12%; H, 4.86%; N, 18.36%. **Compound 4:** yield 61%; mp 172-173^{0}C; IR: 3422, 1597, 1531, 1346 cm^{-1};^{1}H NMR (300MHz,$CDCl_3$) δ 8.18 (1H, s, H-2), 8.08 (1H, s, H-8″), 8.02 (1H, d, H-6″), 7.81 (1H, s, H-8), 7.29 (1H, d, H-5″), 6.46 (1H, br d, OH-2′), 5.81 (1H,
15 d, H-1′), 5.46 (1H, br s, OH-3′), 5.07 (1H, q, H-2′), 4.60-4.47 (3H, br m, OH-5′, H-3′, 1″A), 4.35 (1H, s, H-1″B), 3.94 (1H, d, H-4′), 3.76 (1H, br t, H-5′A), 3.61 (1H, br d, H-5′B), 3.10 (2H, t, H3″ or H4″). Anal. Calcd. For $C_{19}H_{20}N_6O_6$ (428.405): C, 53.27%; H, 4.71%; N, 19.62%. Found: C, 53.21%; H, 4.82%; N, 19.42%. **Compound 5:** yield 66%; mp 148-149^{0}C; MS (ESI) m/z 451 $(M+Na)^+$; IR: 3370, 1590, 1530,
20 1343 cm^{-1}; ^{1}H NMR (300MHz,CD_3COCD_3) δ 8.26 (1H, s, H-2), 8.25 (1H, s, H-8), 7.94 (1H, d, H-7″), 7.60 (1H, d, H-5″), 7.47 (1H, t, H-6″), 5.95 (1H, d, H-1′), 5.78 (1H, br s, OH-2′), 5.63 (1H, d, OH-3′), 4.90 (1H, m, H-2′), 4.64 (2H, br m, OH-5′, H-3′), 4.37 (2H, q, H-4′, 1″A), 4.17 (1H, s, H-1″B), 3.84 (1H, m, H-5′A), 3.71 (1H, m, H-5′B), 3.21 (2H, t, H3″ or H4″). **Compound 22:** yield 95%; mp 157.5-159^{0}C; IR:
25 3413, 1600 cm^{-1}; ^{1}H NMR (300MHz, DMSO-d_6) δ 8.45 (1H, s, H-2), 8.29 (1H, s, H-8), 7.20 (4H, m, H-5″, 6″, 7″, 8″), 5.92 (1H, d, H-1′), 6.47-5.18 (4H, br m, OH-2′, 5′, 3′,H-2′), 4.57 (2H, br m, H-3′, 1″A), 4.14 (1H, q, H-4′), 3.96 (1H, s, H-1″B), 3.67 (1H, m, H-5′A), 3.56 (1H, m, H-5′B), 3.93 (2H, t, H3″ or H4″).

5-NO_2-1, 2,3,4-tetrahydroisoquinoline (6). To a solution of **11** (500mg, 2.85mmole) in acetic acid (50ml) is added small portions of $NaBH_4$ until TLC examination of the reaction mixture indicating the absence of the starting material. The solution is poured onto ice, basified with $NH_3.H_2O$ and then extracted three times
5 with CH_2Cl_2. The organic layer is washed once with water, dried over sodium sulfate and evaporated. The residue is purified by chromatography;(450mg,yield 90%); ^1H NMR (300MHz,$CDCl_3$) δ 7.80 (1H, t, H-7), 7.28 (2H, d, H-6,8), 4.11 (2H, s, $ArCH_2N$), 3.17 (2H, t, $ArCH_2CH_2N$), 3.10 (2H, t, $ArCH_2CH_2N$).

10 **7-NO_2-1, 2,3,4-tetrahydroisoquinline (8).** An ice-cold solution of **12** (10.8g, 80mmol) in concentrated sulfuric acid (40ml) is treated with potassium nitrate (8.8g, 87mmol) in small portions, keeping the temperature below 5 ^0C. The reaction is left overnight at room temperature and poured onto ice. The resulting solution is basified with $NH_3.H_2O$, extracted with CH_2Cl_2 and concentrated to dryness. The crude product
15 obtained is converted to the hydrochloride salt. Crystallization from methanol gave 8.5g of the hydrochloride (yield 50 %), which is basified to give compound **8.** ^1H NMR (300MHz,$CDCl_3$) δ 7.98 (1H, d, H-6), 7.91 (1H, s, H-8), 7.24 (1H, d, H-5), 4.10 (2H, s, H-1), 3.17 (2H, t, H-3), 2.89 (2H, t, H-4).

20 **N-acetyl-7-nitro-1, 2,3,4-tetrahydroisoquinline (13).** A mixture of **8** (5.4g, 26mmol), Et_3N (9.8ml, 70mmol) and acetic anhydride (5.0ml, 51mmol) in CH_2Cl_2 (70ml) is stirred at reflux temperature for 1 hour, cooled, and the reaction mixture is poured onto ice. The resulting solution is extracted three times with methylene chloride. The organic layer is dried over sodium sulfate and evaporated in vacuo. The
25 residue is chromatographed on flash silica gel to give 5.3g of **13** (94% yield). ^1H NMR (300MHz,$CDCl_3$) δ 8.05-8.03 (2H, m, H-6,8), 7.43-7.30 (1H, m, H-5), 4.83 (1.2H, s, $ArCH_2N$), 4.72 (0.8H, s, $ArCH_2N$), 3.87 (0.8H, t, $ArCH_2CH_2N$), 3.74 (1.2H, t, $ArCH_2CH_2N$), 3.02 (1.2H, t, $ArCH_2CH_2N$), 2.95 (0.8H, t, $ArCH_2CH_2N$), 2.21 (1.2H, s, $COCH_3$), 2.20 (1.8H, s, $COCH_3$).

30

N-acetyl-7-amino-1, 2,3,4-tetrahydroisoquinoline (14). A mixture of **13** (5.1g, 23mmol), activated carbon (4.2g), ferric chloride hexahydrate (2.1g, 7.6mmol)

and methanol (140ml) is stirred under refluxing for 20 minutes. To the boiling mixture is added hydrazine hydrate (8.5g, 265mmol) dropwise, and the mixture is refluxed for an additional 4 h, cooled and filtered and the residue washed with methanol. The filtrate is concentrated in vacuo and the residue recrystallized from
5 EtOAc to give compound **14** (3.8g, 85% yield). ^{1}H NMR [300 MHz, $(CD_3)_2SO$], a 3:2 mixture of amide conformers doubling most signals, δ 6.80 (1H, d, H-5), 6.40 (1H, d, H-6), 6.33 (1H, s, H-7), 4.89 (2H, br s, NH_2), 4.46 (0.8H, s, $ArCH_2N$), 4.41 (1.2H, s, $ArCH_2N$), 3.57 (2H, t, ArCH2CH2N), 2.66 (1.2H, t, $ArCH_2CH_2N$), 2.55 (0.8H, t, $ArCH_2CH_2N$), 2.06 (1.2H, s, $COCH_3$), 2.05 (1.8H, s $COCH_3$).
10

N-acetyl-7- trifluoroacetamido-1, 2,3,4-tetrahydroisoquinoline (15). A solution of **14** (3.0g, 17mmol), trifluoroacetic acid (2ml, 28mmol) and trifluoroacetic anhydride (4ml, 28mmol) in CH_2Cl_2 is refluxed for 15 minutes, cooled, and the mixture is poured onto ice, extracted twice with CH_2Cl_2 and evaporated. The mixture
15 is chromatographed on silica gel togive 4.5 g of compound **15** (94% yield). ^{1}H NMR [300 MHz, $(CD_3)_2SO$], a 3:2 mixture of amide conformers doubling most signals, δ 11.24 (0.8H, s, NH), 11.21 (1.2H, s, NH), 7.55-7.40 (2H, m, H5,8), 7.22 (0.6H,s, H-6), 7.20 (0.4H, s, H-6), 4.64 (0.8H, s, $ArCH_2N$), 4.57 (1.2H, s, $ArCH_2N$), 3.65 (2H, t, $ArCH_2CH_2N$), 2.84 (1.2H, t, $ArCH_2CH_2N$), 2.73 (0.8H, t, $ArCH_2CH_2N$), 2.06 (1.2H,
20 s, $COCH_3$), 2.05 (1.8H,s $COCH_3$).

N-acetyl-7-trifluoroacetamido-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (16,17) and N-acetyl-7-amino-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (18,19). Compound **15** (4.5g, 16mol) is dissolved in 30ml ice-cold concentrated H_2SO_4. To
25 this solution is added powdered potassium nitrated (2g, 20mmol) portionwise, keeping the temperature around 4 ^{0}C.The mixture is stirred at 0 ^{0}C overnight, and then poured onto ice. The resulting solution is basified with ammonium hydroxide, and extracted twice with CH_2Cl_2. The organic layer is dried over sodium sulfate and concentrated to dryness. The product is a mixture of compounds **16** and **17** (as
30 determined by ^{1}HNMR). Hydrolysis of compounds **16** and **17** in MeOH (60ml) and concentrated HCl (15ml) at refluxing temperature for 50 min followed by evaporation, basification with ammonium hydroxide and then extracted with CH_2Cl_2.

The organic layer is dried over Na_2SO_4 and evaporated, the crude product is chromatographed on silica gel to give give 1.1g of compound **18** and 0.5g of compound **19**, 30 and 15 % yields, respectively. Compound **16**: MS (ESI) m/z 354 $(M+Na)^+$, m/z 330 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide
5 conformers doubling most signals, δ 11.38 (0.4H, s, NH), 11.32 (0.6H, s, NH), 8.56 (1H, s, H-5), 8.15 (0.4H, s, H-8), 8.13 (0.6H, s, H-8), 4.86 (1.2H, s, $ArCH_2N$), 4.74 (0.8H, s, $ArCH_2N$), 3.88 (0.8H, t, $ArCH_2CH_2N$), 3.74 (1.2H, t, $ArCH_2CH_2N$), 2.99 (1.2H, t, $ArCH_2CH_2N$), 2.93 (0.8H, t, $ArCH_2CH_2N$), 2.21 (3H, s, $COCH_3$). Compound **17**: MS (ESI) m/z 354 $(M+Na)^+$, m/z 330 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$), a 3:2
10 mixture of amide conformers doubling most signals, δ 9.64 (1H, br s, NH), 8.21 (0.4H, d, H-5), 8.18 (0.6H, d, H-5), 7.46 (0.4H, d, H-6), 7.43 (0.6H, d, H-6), 4.89 (1.2H, s, $ArCH_2N$), 4.75 (0.8H, s, $ArCH_2N$), 3.86 (0.8H, t, $ArCH_2CH_2N$), 3.78 (1.2H, t, $ArCH_2CH_2N$), 3.01 (1.2H, t, $ArCH_2CH_2N$), 2.96 (0.8H, t, $ArCH_2CH_2N$), 2.06 (3H, s, $COCH_3$). Compound **18**: MS (ESI) m/z 258 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$),
15 a 2:1 mixture of amide conformers doubling most signals, δ 7.94 (1H, s, H-5), 6.60 (1H, s, H-8), 5.96 (2H, br s, NH_2), 4.68 (1.3H, s, $ArCH_2N$), 4.56 (0.7H, s, $ArCH_2N$), 3.80 (0.7H, t, $ArCH_2CH_2N$), 3.67 (1.3H, t, $ArCH_2CH_2N$), 2.85 (1.3H, t, $ArCH_2CH_2N$), 2.79 (0.7H, t, $ArCH_2CH_2N$), 2.18 (3H, s, $COCH_3$); ^{13}C NMR δ 169.9 and 169.7 ($COCH_3$), 143.6 and 143.2, 142.5 and 141.5, 131.5 and131.3, 123.4 and
20 123.5 (C-6, 7,9,10), 126.1 and 125.5, 116.1 and 115.5 (C-5,8), 48.1, 44.4, 44.1, 39.9 (C-1,3), 28.5 and 27.5 (C-4), 22.1 and 21.8 ($COCH_3$). Compound **19**: MS (ESI) m/z 258 $(M+Na)^+$, m/z 234 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 7.10 (0.6H, d, H-5), 7.05 (0.4H, d, H-5), 6.73 (0.6H, d, H-6), 6.68 (0.4H, d, H-6), 5.52 (1.2H, s, NH2), 5.34 (0.8H, s, NH_2), 4.93
25 (0.8H, s, $ArCH_2N$), 4.81 (1.2H, s, $ArCH_2N$), 3.79 (1.2H, t, $ArCH_2CH_2N$), 3.68 (0.8H, t, $ArCH_2CH_2N$), 2.83 (0.8H, t, $ArCH_2CH_2N$), 2.77 (1.2H, t, $ArCH_2CH_2N$), 2.16 (3H, s, $COCH_3$). ^{13}C NMR δ 169.5 and 169.2 ($COCH_3$), 142.9 and 142.4, 131.0 and 130.1, 125.7, 124.3 (C-7, 8,9,10), 135.4 and 134.5, 117.7 and 117.2 (C-5, 6), 47.3, 43.7, 42.9, 39.1 (C-1, 3), 29.0 and 28.1 (C-4), 22.1 and 21.6 ($COCH_3$).

30

N-acetyl-6 (8)-nitro-1, 2,3,4-tetrahydroisoquinoline (20,21). To a stirred solution of compound **18** **(19)** (290mg, 1.23mmol) in HCl (6M, 4ml) is added dropwise a solution of $NaNO_2$ (110mg, 1.6 mmol) in water (0.5 ml) at 0 °C. After stirring at 0 °C for 2h, hypophosphorous acid (50% aqueous solution, 1.4ml) is added
5 dropwise, and the mixture is stirred at 40 °C for 10h, then poured into water and extracted with methylene chloride. The organic layer is dried over $NaSO_4$ and evaporated. The crude product is purified by chromatography and afforded compound **20** **(21)** (233mg, 86%). Compound **20**: MS (ESI) m/z 243 $(M+Na)^+$, m/z 219 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$), a 2:1 mixture of amide conformers doubling most
10 signals, δ 8.08-8.03 (2H, m, H-5, 7), 7.31 (1H, d, H-8), 4.83 (1.3H, s, $ArCH_2N$), 4.72 (0.7H, s, $ArCH_2N$), 3.87 (0.7H, t, $ArCH_2CH_2N$), 3.74 (1.3H, t, $ArCH_2CH_2N$), 3.02 (1.3H, t, $ArCH_2CH_2N$), 2.96 (0.7H,t, $ArCH_2CH_2N$), 2.21 (3H, s, $COCH_3$). Compound **21**: MS (ESI) m/z 243 $(M+Na)^{+;}$ ^1H NMR (300 MHz, $CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 7.97 (0.6H, d, H-7), 7.94 (0.4H, d, H-7), 7.49-
15 7.32 (2H, m, H-6, 5), 5.09 (0.8H, s, $ArCH_2N$), 4.99 (1.2H, s, $ArCH_2N$), 3.86 (1.2H, t, $ArCH_2CH_2N$), 3.75 (0.8H, t, $ArCH_2CH_2N$), 3.03 (0.8H, t, $ArCH_2CH_2N$), 2.98 (1.2H, t, $ArCH_2CH_2N$), 2.21 (1.8H, s, $COCH_3$), 2.19 (1.2H, s, $COCH_3$).

6(8)-nitro-1, 2,3,4-tetrahydroisoquinoline (6 and 8). Hydrolysis of **20** **(21)**
20 (190 mg, 0.86 mmol) in MeOH (6 ml) and concentrated hydrochloride acid (3ml) at refluxing temperature for 10 h afforded 6(8)-nitro-1, 2,3,4-tetrahydroisoquinolines (100mg, 66%), which are separated by flash silica gel chromatography 6-NO_2-1, 2,3,4-tetrahydroisoquinoline (**20**): MS (ESI) m/z 179 $(M+H)^+$; ^1H NMR (300 MHz, CDC_{l3}) δ 7.99-7.96 (2H, m, H-5, 7), 7.16 (1H, d, H-8), 4.10 (2H,s, $ArCH_2N$), 3.17
25 (2H, t, $ArCH_2CH_2N$), 2.90 (2H, t, $ArCH_2CH_2N$). 8-NO2-1, 2,3,4-tetrahydroisoquinoline (**21**): MS (ESI) m/z 179 $(M+H)^{+;}$ ^1H NMR (300 MHz, CD_3OD) δ 7.80 (1H, d, H-7), 7.44 (1H, d, H-5), 7.33 (1H, t, H-6), 4.16 (2H, s, $ArCH_2N$), 3.07 (2H, t, $ArCH_2CH_2N$), 2.92 (2H, t, $ArCH_2CH_2N$).

30 **N-acetyl-6, 8-dinitro-7-trifluoroacetamido-1, 2,3,4-tetrahydroisoquinoline:** MS (ESI) m/z 399 $(M+Na)^+$, m/z 375 $(M-H)^{-;}$ IR: 3448, 1640, 1647, 1270, 1171 cm^{-1}; 1H NMR (300 MHz, DMSO-d_6), a 3:1 mixture of

amide conformers doubling some signals, δ 7.74 (1H, s, H-5), 4.48 (2H, s, $ArCH_2N$), 3.70 (2H, t, $ArCH_2CH_2N$), 2.90 (1.5H, t, $ArCH_2CH_2N$), 2.78 (0.5H, t, $ArCH_2CH_2N$), 2.07 (2.3H, s, $COCH_3$). 2.07 (0.7H, s, $COCH_3$).

5 Example 4 - Biological Testing

Exemplary compounds are tested to determine their *es* nucleoside transporter binding ability by a flow cytometric assay (Buolamwini *et al.*, 1994). Human leukemia K562 cells growing in RPMI 1640 medium are washed once and suspended at 1.6 x 10^6 cells/ml in phosphate-buffered saline at pH 7.4, and incubated with 5-
10 (SAENTA)-*X8*-fluorescein (25 nM) in the presence or absence of varying concentrations of test compounds at room temperature for 45 minutes. Flow cytometric measurements for cell-associated fluorescence are then performed with a FACSCalibur (Becton Dickinson, San Jose, CA) equipped with a 15 mW-argon laser (Molecular Resources Flow Cytometry Facility, University of Tennessee Health
15 Sciences Center). In each assay, 5,000 cells are analyzed from suspensions of 4 x 10^5 cells/ml. The units of fluorescence are arbitrary channel numbers. Percentage (%) of control (i.e. *es* transporter-specific fluorescence in the presence of SAENTA-fluor without test compounds) is calculated for each sample by the equation below.

20 $$\% \text{ Control} = \frac{(SF_s)}{(SF_f)} \times 100 \qquad \text{Eq. 1}$$

where SF_s is the *es* transporter-specific fluorescence of test samples, and SF_f is the *es* transporter-specific fluorescence of the SAENTA-fluor
25 ligand standard.

The results are fed into the PRISM program (GraphPad, San Diego, CA) to derive concentration-dependent curves using nonlinear regression, as shown in Figure 1. From these curves, the IC_{50} values are obtained that are used to compare abilities of
30 the new compounds to displace the *es* transporter-specific ligand (5-(SAENTA)-*X8*-fluorescein (Buolamwini *et al.*, 1994).

Examples of the present invention (**2-5**) are tested along with 6-(1,2,3,4-tetrahydroisoquinolinyl)purine riboside (**23**) as *es* transporter binding ligands by a facile competitive binding flow cytometric assay using the K562 chronic myelogenous leukemia cell line. The high-affinity *es* transporter fluorescent ligand,
5 5-(SAENTA)-*X8*-fluorescein is used as the competitive ligand to be displaced by the test compounds. Flow cytometry has several advantages over the conventional radioligand binding assays, in that it eliminates radiation hazards and disposal problems, and allows the use of much fewer cells, as few as 5,000 cells compared to 2 million cells per sample for comparable radioligand assays. The compounds of the
10 present invention exhibit a wide range of binding affinities at the *es* transporter as measured by their ability to displace the *es* nucleoside transporter-specific ligand, shown by the K_i values in Table 1.

Compound	K_i (nM)
1 (NBMPR)	0.70
2	250
3	15
4	0.45
5	300
23	150

Table 1. Flow Cytometrically-Determined K_i Values

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They range from a subnanomolar concentration for compound **4** (K_i 0.45 nM) to high nanomolar concentrations for compounds **2** and **5**, at least about a 500 fold difference. The results indicate that, based on known SAR of NBMPR analogs, the compounds of the present invention are as good or even possibly better than the prototype *es*
20 transporter inhibitor NBMPR. The comparable affinities of compound **4** (K_i 0.45 nM) and NBMPR (K_i 0.70 nM) indicate that conformational changes at this location in the molecules can make a significant difference in binding to the *es* transporter. The corresponding *para* compound **3**, is tightly bound (K_i 15 nM), but much less so than the compound corresponding to the *meta* nitro substitution, compound **4** (K_i 0.45
25 nM). These results offer insights into the bioactive conformation of NBMPR at the 6-position substituent, which is responsible for high affinity binding to the *es* transporter. The solid state conformation of NBMPR has been determined by X-ray

diffraction and a solution conformation has also been determined by NMR. The X-ray structure reveals a *syn* orientation of the purine system about the glycosidic bond, whereas the solution conformation reveals the preponderance of a high-anti orientation of the purine system about the glycosidic linkage. Conformational space analysis of the novel conformationally constrained analogs reported here will shed light on the bioactive conformation of NBMPR and provide insights for further *es* transporter inhibitor design.

The *es* transporter binding high affinity of compound **4,** which is at least equivalent in potency to NBMPR, is of interest since this compound will be less likely to lose the nitrobenzyl moiety, and should overcome a major disadvantage of NBMPR, which has to do with the *in vivo* lability of the nitrobenzyl moiety. The nitrobenzyl group of NBMPR is lost *in vivo* to form the immunosuppressive product mercaptopurine riboside, with a dramatically reduced *es* transporter affinity (up to a 1000 fold decrease). This lability of the nitrobenzyl group has actually allowed NBMPR to be used as an effective photolabeling agent of the *es* nucleoside transporter. This has hampered the clinical applications of NBMPR. Therefore preclinical development of the novel compounds **3** and **4**, which lock the nitrobenzyl moiety in place, and are likely to prolong *in vivo* activity compared to NBMPR, is warranted. Compounds **3** and **4** represent the Best Mode.

Example 5 – Inhibition of Uridine Uptake

Cells in logarithmic phase of growth are incubated with 1 μM [^{3}H]uridine and 10-second uptake measurements are performed as described in Paterson *et al.* (1983). Figure 2 indicates that compound **4** potently inhibits the uptake of uridine, a universal nucleoside transporter substrate, better than the prototype standard compound NBMPR, with IC_{50} value in the single digit nanomolar range. These uptake results reflect the flow cytometric binding assay results (see Fig. 1), which show that compound **4** binds very tightly at the *es* transporter.

Example 7 – Heart Treatment During Ischemia

Results shown in Table 2 indicate that both compound **4** and the standard agent NBMPR do enhance the recovery in the rat heart isolated model, indicating that

compound **4** is a cardioprotective agent. As shown in Table 2, NBMPR doubles the percentage recovery from control levels, compound **4** triples recovery from control levels. In addition, treatment with compound **4** also appears to keep the heart more relaxed than treatment with either control or NBMPR, an added benefit not seen with
5 the standard agent.

Table 2: Effect of Compounds on Rat Heart Recovery from Global Ischemia:

Heart Treatment during Ischemia and Reperfusion	**Recovery from 30 min Global Ischemia (LVDP)[a]**	**Change in EDP[b]**
Control (0.02% DMSO)	12.03 ± 1.7 % (n = 6)[c]	66.4 ± 3.0 %
NBMPR (Standard agent)	24.40 ± 3.6 % (n = 5)[d]	65.2 ± 3.2 %
Compound **4** (New agent)	33.60 ± 9.8 % (n = 4)[e]	52.5 ± 6.6 %[f]

10 [a]LVDP = left ventricular developed pressure. [b]EDP = end diastolic pressure. [c]Data points are the mean SEM, and n is number of animals used. [d]Statistically significant at $p < 0.01$. [e]Statistically significant at $p < 0.03$. [f]Statistically significant in one tail test; $p < 0.04$.

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Throughout the disclosure and attachment, various patents and/or publications are referenced, specifically including the lists below. All such patents and/or publications are expressly incorporated herein by reference in their entirety and as such are considered part of this disclosure.

25

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20

The following patents relate to the field of nucleoside transport inhibitors within the scope of certain embodiments of the present invention, and are incorporated herein by reference:

6,455,507 Benzimidazole derivatives;

25 6,455,506 Lyxofuranosyl benzimidazoles as antiviral agents;

6,423,829 Nitrobenzylmercaptopurineriboside (NBMPR)-insensitive, equilibrative, nucleoside; transport protein, nucleic acids encoding the same and methods of use;

6,413,938 Benzimidazole derivatives for the treatment of viral infections;

30 6,403,589 Method of treating pain with draflazine-analogues;

6,350,753 2-Substituted-4-substituted-1,3-dioxolanes and use thereof ;

6,342,501 Pyrrolo[2,3-d] pyrimidines as antiviral agents;

6,319,946 Inhibitors of aspartyl protease;
6,307,043 Benzimidazole and its ribonucleoside;
6,297,250 Pyrimidopyrimidine compounds;
6,225,460 Nucleotide analogs;
5 6,180,639 1,3-oxathiolane nucleoside analogues;
6,177,435 Therapeutic combinations;
6,156,737 Use of dideoxy nucleoside analogues in the treatment of viral infections;
6,096,786 Immunopotentiatory agent and physiologically acceptable salts
10 thereof;
5,998,605 Antiviral benzimidazole nucleoside analogues and method for their preparation;
5,977,061 N_6 - substituted nucleotide analagues and their use;
5,958,980 Immunopotentiatory agent and physiologically acceptable salts
15 thereof;
5,922,696 Ethylenic and allenic phosphonate derivatives of purines;
5,905,082 Crystalline oxathiolane derivatives;
5,886,179 Nucleotide analogs;
5,872,151 Immunopotentiatory agents and physiologically acceptable salts
20 thereof;
5,817,647 Unsaturated acetylene phosphonate derivatives of purines;
5,798,340 Nucleotide analogs;
5,767,100 Compounds and methods for making and using same;
5,750,729 Compounds and methods for making and using same;
25 5,717,095 Nucleotide analogs;
5,693,771 Methods for making nucleoside analogs;
5,681,581 Controlled release pharmaceutical formulations of 3'-azido-3'-deoxythymidine and methods of use;
5,670,520 Method for inhibiting virus replication in mammalian cells using
30 carbostyil derivatives;
5,663,154 2',3'-dideoxy-3'-fluoro-purine ribonucleosides;
5,659,023 Nucleotide analogues;

5,656,745 Nucleotide analogs;

5,618,820 1,3-oxathiolane nucleoside analogues and methods for using same;

5,607,929 Antiviral dibenzothiazepinone derivatives;

5,589,474 Dibenzothiazepinthione as antiviral agents;

5 5,587,480 Substituted 1,3-oxathiolanes and substituted 1,3-dithiolanes with antiviral properties;

5,574,149 Method of treating HIV infections with 2',3'-dideoxy-3'-fluoro-5-chlorouridine;

5,547,976 Further indole derivatives with antiviral activity;

10 5,538,975 1,3-oxathiolane nucleoside compounds and compositions; and

5,508,310 Immunopotentiatory agents and physiologically acceptable salts thereof

5,504,093 Method for inhibiting nucleoside and nucleobase transport in mammalian cells, and method for inhibition of DNA virus replication

15 5,491,135 Compositions of N-(phosphonoacetyl)-L-aspartic acid and methods of their use as broad spectrum antivirals

It will be apparent to one of ordinary skill in the art that various modifications and variations can be made in the present invention without departing from the spirit
20 and scope of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. The above examples and preferred embodiments are for exemplary purposes, and not intended to limit the spirit and scope of the present invention.

25 Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, and so forth used herein are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that may vary depending upon the desired properties sought to be determined by the present
30 invention.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations (particularly in the disclosure, above), the numerical values set forth in the disclosure / experimental or example sections are reported as precisely as possible. Any numerical value, however,
5 inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

I claim:

1. A compound of the following formula, and analogs thereof:

5 FORMULA I

wherein:

X_1-X_9 are each independently C, N, provided that where X is N, the R substituent is absent;

Y is O, S, NH, CH_2;

10 R_1 is H, OH, NH_2, N_3, halogen;

R_2 is H, OH, NH_2, N_3, halogen;

R_3 is H, NH_2, substituted amino, halogen;

R_4 is H, OH, NO_2, CN, N_3, NCS, halogen;

R_5 is H, OH, NO_2, CN, N_3, NCS, halogen, halogen-CH_2COCH_3;

15 R_6 is H, OH, NO_2, CN, N_3, NCS, halogen, halogen-CH_2COCH_3;

R_7 is H, OH, NO_2, CN, N_3, NCS, halogen;

R_9 is H, OH, NH_2, N_3, halogen;

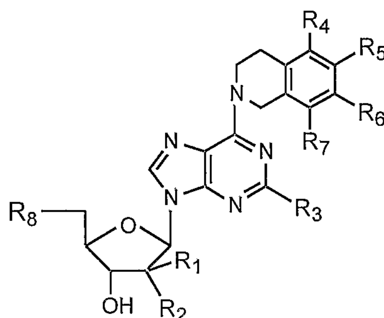
R_8 is H, OH, OCH_3, NO_2, CN, N_3, NCS, halogen, A-Reporter (where A is O, S, NH, and the Reporter is a tethered reporter group;

20 R_{10} is H, OH, NH_2, N_3, halogen;

provided that when X_1-X_4, X_6, are all C, X_5, X_7, X_8, X_9 are all N, and Y is O, substituents R_4, R_5, R_6, R_7, cannot all be H at the same time if R_1 is H, R_2 is OH, R_3 is H and R_8 is OH.

5

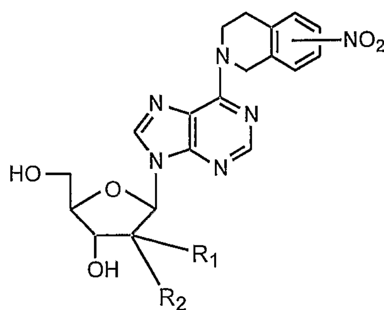
2. A compound of claim 1, of the following formula, and analogs thereof:



,

10 wherein R_1-R_8 are defined as in claim 1.

3. A compound of claim 1, of the following formula, and analogs thereof:

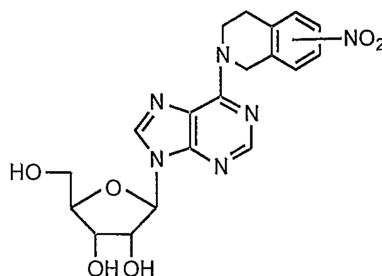


,

15

wherein R_1-R_2 are defined as in claim 1.

4. A compound of claim 1, of the following formula, and analogs thereof:



.

5. A method of inhibiting a nucleoside transporter in a subject in need thereof,
5 comprising:

administering a nucleoside transporter inhibiting amount of a compound of one of claim 1-4, pharmaceutically acceptable acid addition salt, ester, or prodrug thereof of the following formula to the subject.

10 6. A method of preventing or treating heart disease or stroke conditions in a subject in need thereof, comprising:

administering a nucleoside transporter inhibiting amount of a compound of one of claim 1-4, pharmaceutically acceptable acid addition salt, ester, or prodrug thereof of the following formula to the subject.

15

7. The method of claim 6, wherein the heart disease is ischemic heart disease.

8. A method of preventing or treating infectious disease in a subject in need thereof, comprising:

20 administering a nucleoside transporter inhibiting amount of a compound of one of claim 1-4, pharmaceutically acceptable acid addition salt, ester, or prodrug thereof of the following formula to the subject.

9. The method of claim 8, wherein the infectious disease is a viral infection.

25

10. The method of claim 8, wherein the infectious disease is an HIV-related disease.

11. The method of claim 8, wherein the infectious disease is a gram-positive bacterial infection.

5 12. The method of claim 8, wherein the infectious disease is a gram-negative bacterial infection.

13. The method of claim 8, wherein the infectious disease is a fungal infection.

10 14. The method of claim 8, wherein the infectious disease is a protozoal infection.

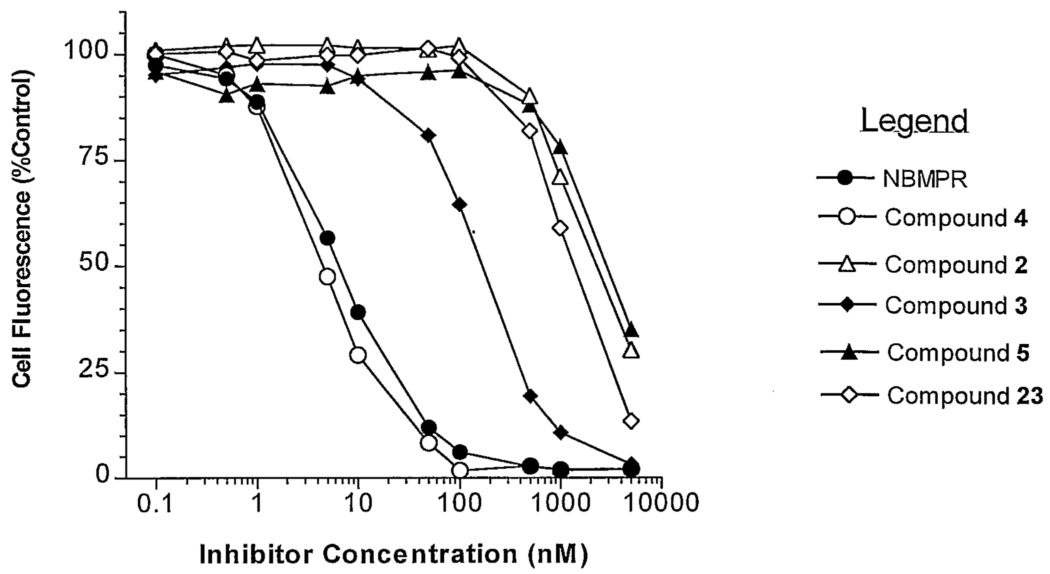


Fig. 1

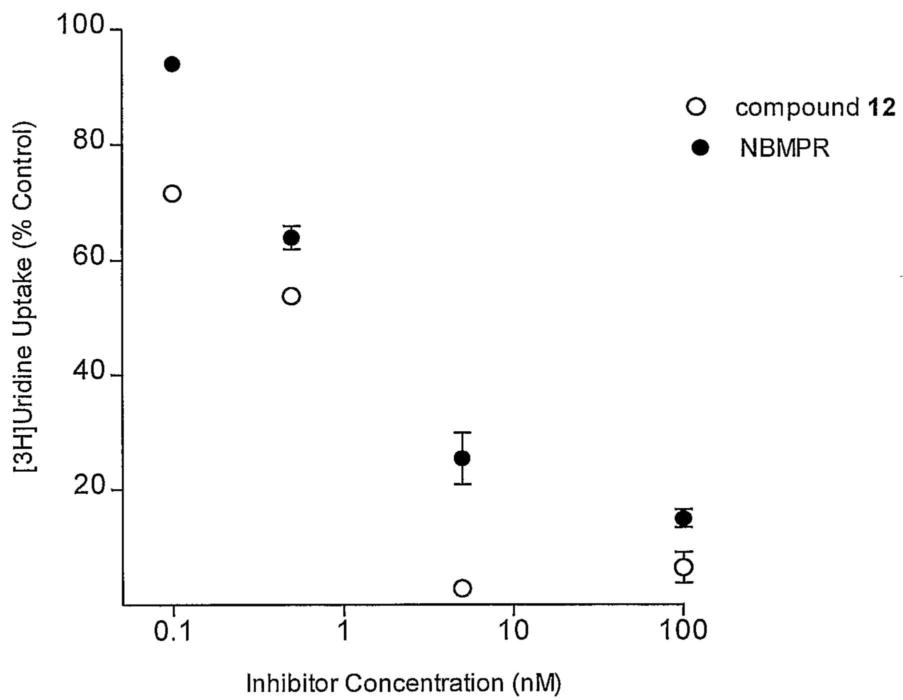


Fig. 2