Pergamon

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Bioorganic & Medicinal Chemistry 11 (2003) 899–908

BIOORGANIC & MEDICINAL CHEMISTRY

Inhibition of Nucleoside Transport By New Analogues of Nitrobenzylthioinosine

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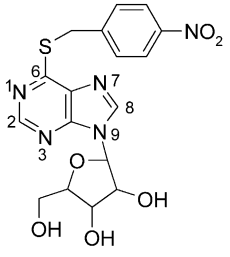
Received 26 July 2002; accepted 22 October 2002

Abstract—Nitrobenzylthioinosine (NBTI, **1**) was systematically modified by attachment of substituents at positions C6 and N9, and also by substitution of N1 with C. These modifications were chosen to reduce the polarity of the new compounds. Incorporation of the nitro functionality into a benzoxadiazole ring system was considered first. These new nucleosides showed high affinity (1.5–10 nM) towards the nucleoside transport protein as present on human erythrocyte ghosts. Next, modification of this benzoxadiazole ring system with C, S and O in different positions produced a number of less polar nucleosides with affinity in the higher nanomolar range. Modification of N9 was achieved with different alkyl and alcohol substituents. An *n*-butyl substituent proved best, although all variations yielded substantial decreases in affinity. Replacement of N1 by a carbon atom in combination with a 2-Cl substituent also resulted in a relatively potent NBTI derivative (47 nM).

Introduction

The facilitated, carrier-mediated transport of nucleosides across mammalian cell membranes can be inhibited by a number of ligands including nucleoside derivatives such as nitrobenzylthioinosine (NBTI, **1**),[1] and non-nucleoside compounds including marketed substances such as dipyridamole and dilazep.[2,3]



NBTI, 1

Such compounds contribute to the physiological actions of adenosine. Through their blockade of the transport protein they increase the extracellular concentration of adenosine. This increase in adenosine levels causes a more profound occupancy of adenosine receptors through which adenosine exerts many of its physiological effects. The high hydrophilicity of NBTI and other transport inhibitors, however, may hinder their penetration into the CNS, where adenosine is involved in, for example, counteracting neuropathic pain.

An earlier study indicated that a nitro group preferably at the 4-position of the benzyl moiety in NBTI is a prime factor in determining the potency of inhibition of nucleoside transport in human erythrocytes.[4,5] To gain further information on the interaction of NBTI with the nucleoside transporter-associated binding site we systematically replaced a number of substituents at C6 and N9. Also the 1-deaza-2-chloro analogue of NBTI was prepared. The prime aim was to provide substances with reduced polarity while maintaining substantial affinity for the transport protein.

Results

Chemistry

The first modification was conversion of the nitro group in the 4-nitrobenzyl moiety to an oxadiazole ring system

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PII: S0968-0896(02)00544-8

(**14–16**, Scheme 1). This is a cyclic functionality, which resembles the nitro group. Cyclocondensation of the appropriate azide (**2–4**) in boiling toluene gave benzofuroxanes (**5–7**).[6] Refluxing in ethanol in the presence of triethylphosphite resulted in deoxygenation to benzofurazanes **8–10**. Benzylic bromination of these compounds was performed in the presence of *N*-bromosuccinimide (NBS) and benzoyl peroxide. Finally, coupling of the resulting benzylic bromides **11–13** with 6-mercaptopurine riboside, affording compounds **14–16**, was achieved in DMF at room temperature.[8]

The next series of analogues of NBTI were compounds **23** and **24** (Scheme 2). These deaza analogues of the benzoxadiazole substituents were obtained by coupling bromomethyl-2,1-benzisoxazole with 6-mercaptopurine riboside. Bromides **21** and **23** were synthesized by ring closure of the appropriate *ortho*-nitrobenzaldehyde (**17–18**) in the presence of tin(II)chloride and subsequent bromination with NBS.[9]

2,1-Benzoisothiazole analogues **28** and **31** were obtained via a general procedure, which involved cyclization of 2,4-dimethylaniline with *N*-sulfinylmethane sulfonamide (Scheme 3).[10] Treatment of **26** with *n*-BuLi and bromine gave compound **29**. Benzylic bromination of **26** and **29** with NBS followed by coupling with 6-mercaptopurine riboside afforded analogues **28** and **31**.

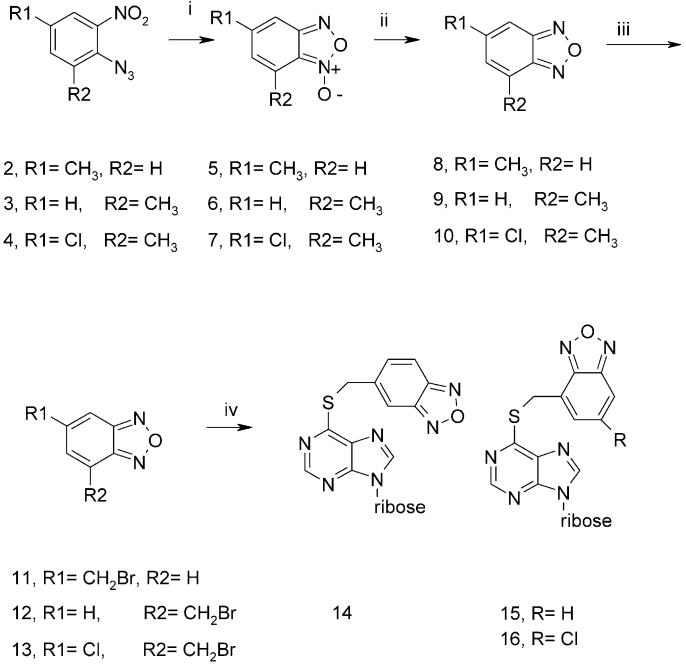
Benzimidazole analogues **37** and **38** were synthesized by a Mitsunobu coupling[11] of 6-mercaptopurine riboside with benzimidazole-methyl alcohol,[12] in the presence of (cyanomethyl)trimethylphosphonium iodide (**36**) and diisopropylethylamine (DIPEA) in propionitrile (Scheme 4). After refluxing of the mixture for 18 h, water was added and the product precipitated. The direct use of alcohols **34** and **35** is an advantage of this method compared to the previous reactions.[8] These alcohols were obtained from a reduction of the appropriate esters via standard procedures.[13]

Benzothiazole **41** was prepared as shown in Scheme 5. Ethyl benzothiazole-6-carboxylate **39** was obtained from the ring closure of ethyl 4-amino-3-mercaptobenzoate.[14] Reduction of this ester under standard conditions at 0 °C gave alcohol **40**.[15] Mitsunobu coupling of 6-mercaptopurine riboside with **40** afforded nucleoside **41** in 56% yield.

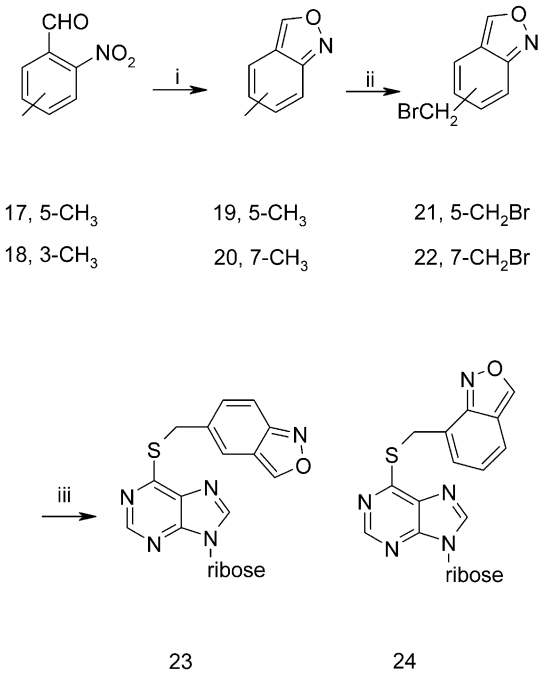
For the alkylation of the N9 position three methods were applied. Reaction of 6-substituted mercaptopurine **42** with 2,3-dihydrofuran or 2,3-dihydro-4*H*-pyran in the presence of a catalytic amount of *p*-toluenesulfonic acid led to **43** and **44**, respectively (Scheme 6). Such compounds have been described as purine deoxynucleosides.[16]

Direct introduction of an alkyl moiety to purine bases at the N9 position was achieved by a Mitsunobu reaction of the proper alcohol and 6-substituted purine **42**.[17] One example of such an alkylation is illustrated in Scheme 7. Diethyl azodicarboxylate was added in portions to a mixture of purine **42**, alcohol **45**,[18] and triphenylphosphine in THF. Since the substituent on C6 is large the main product is the N9 substituted purine **46**. Deprotection of the resulting compound with standard procedures gave compound **47**. The same method was used for preparation of the N9-cyclopentyl substituted compound **48**.

For substitution of N9 with an *n*-butyl group, a solution of the appropriate nucleobase in dry DMF was added



Scheme 1. Preparation of benzofurazan analogues: (i) toluene, reflux, 2 h; (ii) $P(OEt)_3$, ethanol, reflux, 30 min; (iii) NBS, Bz_2O_2, CCl_4, N_2, reflux, 18 h; (iv) 6-mercaptopurine riboside, K_2CO_3, DMF, rt.



Scheme 2. Preparation of benzisoxazole analogues: (i) $SnCl_2$, $2H_2O$, HCl, 15 °C, 2 h; (ii) NBS, Bz_2O_2, CCl_4, N_2, reflux, 12 h; (iii) 6-mercaptopurine riboside, K_2CO_3, DMF, 2 h.

dropwise to a suspension of sodium hydride in DMF (Scheme 8). After 30 min, to ensure the complete formation of the anion, *n*-butyl bromide was added in excess to give the desired N9-butylated derivatives (**53**–**56**) with small amounts of the N7 isomers that were removed by column chromatography.

Modification of N1 in the purine ring system was achieved by preparing the 1-deaza-2-chloropurine analogue of NBTI. The synthesis of 1-deaza-2-chloropurine has been previously reported.[19] Acetate-protected 2,6-dichloro-1-deazapurine riboside **57** was coupled with benzyl mercaptan in the presence of triethylamine. Deprotection of the ribose moiety with a saturated solution of ammonia in methanol gave the proper nucleoside (Scheme 9).

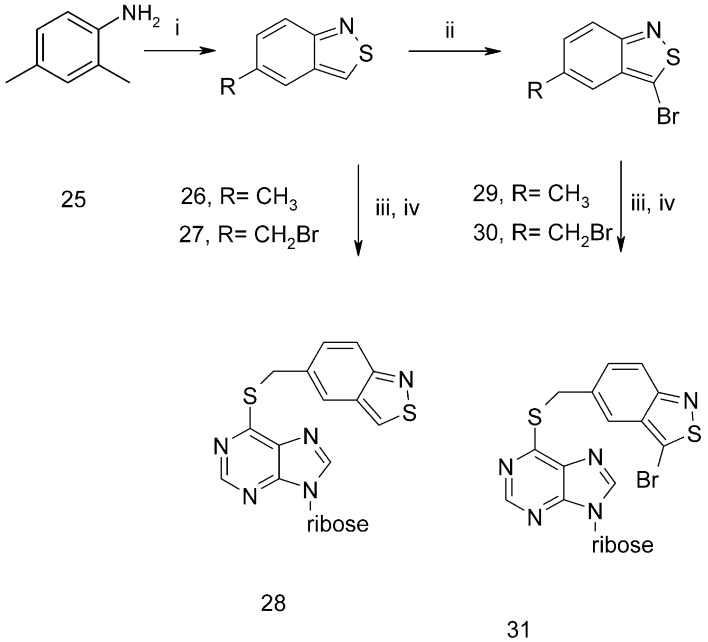
Biological studies

All final products were tested in a radioligand binding assay.[3,20] Human erythrocyte membranes were used as a rich source of the nucleoside transport protein, with [^{3}H]NBTI as the radioligand (K_D value: 0.59 ± 0.07 nM). In Table 1, the results are shown for the inhibition by NBTI and the various NBTI derivatives of the equilibrium binding of [^{3}H]NBTI to human erythrocytes.

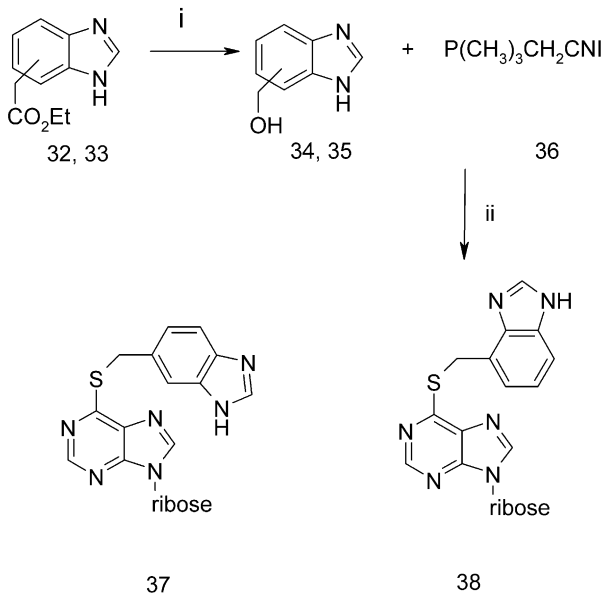
Discussion

The aim of the first series of modifications was conversion of the 4-nitrobenzyl group on NBTI to a substituent with a lower hydrophilicity. The oxadiazole functionality instead of a nitro group in compounds **14**–**16** showed high activity, comparable to NBTI itself. This ring system was then systematically modified to yield benzo-2,1-isoxazole (**23** and **24**), benzo-2,1-isothiazole (**28** and **31**), benzimidazole (**37**, **38**) and benzothiazole (**41**) derivatives, respectively.

Compound **23** showed reduced activity compared to **14**. Apparently, replacement of one N with C as in compound **23** is not very favorable, although such manipulation substantially decreases the compound's hydrophilicity. In a further effort to reduce polarity, S was introduced instead of O in the isoxazole ring to



Scheme 3. Preparation of benzoisothiazole analogues: (i) CH_3SO_2NSO, benzene (dry), 0 °C to reflux 18 h; (ii) *n*-BuLi, THF (dry), −78 °C, Br_2, −78 °C to rt; (iii) NBS, Bz_2O_2, CCl_4, N_2, reflux, 12 h; (iv) 6-mercaptopurine riboside, K_2CO_3, DMF, 2 h.



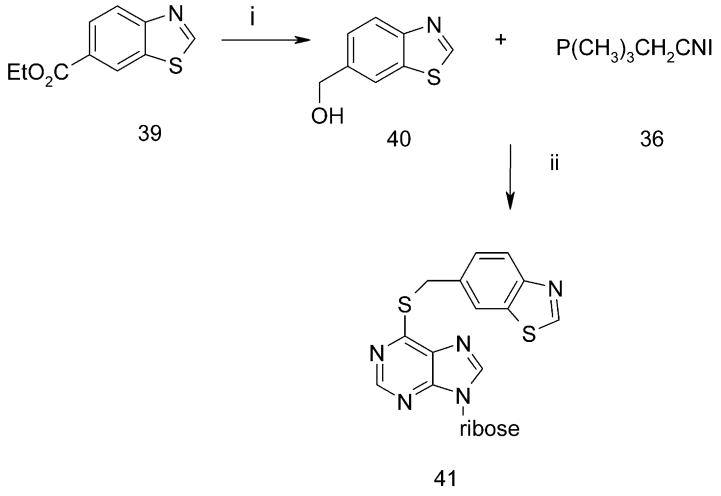
37 38

Scheme 4. Preparation of benzimidazole analogues: (i) $LiAlH_4$/THF, 18 h, reflux; (ii) 6-mercaptopurine riboside, DIPEA, propionitrile, reflux, 18 h.

produce isothiazole **28**. Also this compound showed a decline in affinity, with both compounds displaying K_i values of approx. 100 nM.

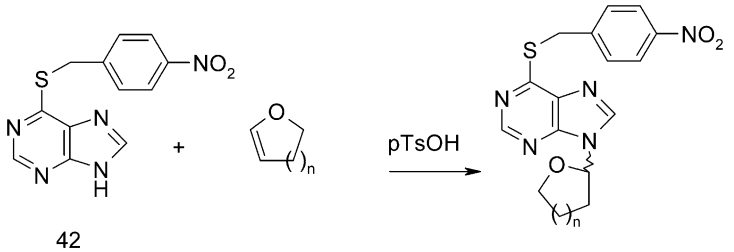
Further modification of the oxadiazole functionalities in **14** and **15** to imidazoles (**37** and **38**) had a negative effect on affinity. Therefore, thiazole **41,** with S instead of NH in the corresponding imidazole moiety, was prepared. This substantially less polar derivative had appreciable affinity for the nucleoside transport protein ($K_i = 165$ nM), although quite comparable to imidazole derivative **37**.

In the C6-substituted compounds we also studied the effect of the position of substitution with respect to the $–SCH_2–$ bridge between nucleobase and ring substituent (pairs of compounds **14**–**15**, **23**–**24** and **37**–**38**). All '*ortho*' substituted nucleosides (**15**, **24** and **38**) showed a reduced affinity when compared to their counterparts. A similar conclusion was drawn in another study in which the nitro functionality was shifted from the *para* position to other positions in the ring.[4] Further modification of compound **15** by introduction of chlorine in the ring system did not improve the binding affinity in **16**. An analogous approach, bromination of **28** to yield **31**, resulted in a 2.6-fold affinity gain from 108 to 42 nM.



41

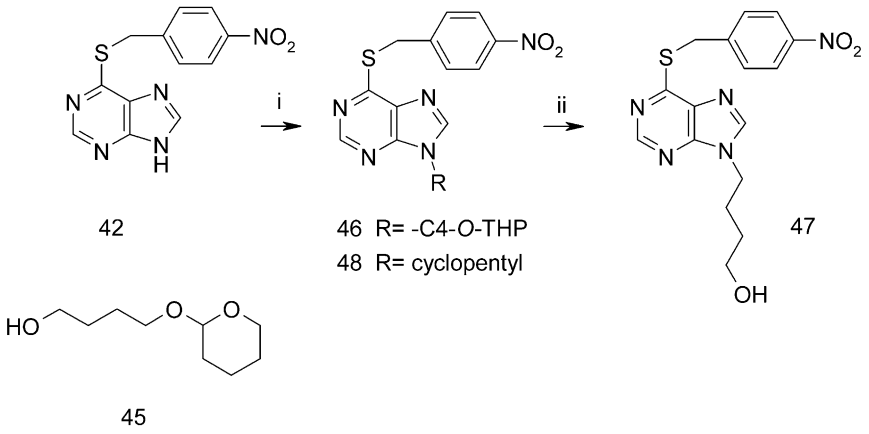
Scheme 5. Preparation of benzothiazole analogues: (i) $LiAlH_4$/THF, 18 h; (ii) 6-mercaptopurine riboside, DIPEA, propionitrile, reflux, 18 h.



42

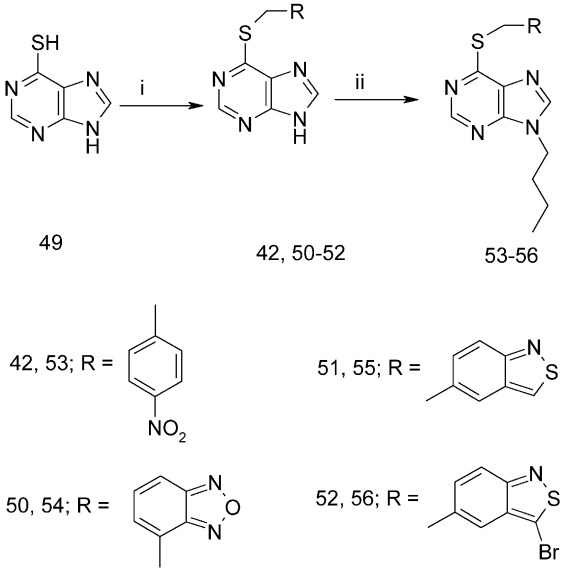
43 n= 1
44 n= 2

Scheme 6. Synthesis of THF- and THP-protected compounds.



Scheme 7. Synthesis of N9-alkyl-substituted purines via a Mitsunobu reaction: (i) DEAD, PPh_3, THF (dry), **45** or cyclopentanol; (ii) pTsOH, rt, 2 h.

Next, the effect of N9 substitution was studied. Since the ribose group with three hydroxyl groups is very hydrophilic, the N9 position was substituted with different alkyl and modified alkyl groups. Introduction of a hydroxyl group at C4 in a butyl substituent (**47**) had a negative effect on the binding affinity, whereas the protected alcohol showed a higher affinity (**46**). Compound **48** with a cyclopentyl group on N9 showed a decrease in activity compared to a simple n-butyl substituent (**53**). Compounds **43** and **44** bearing a tetrahydrofuran and tetrahydropyran N9-substituent, respectively, were designed as deoxy sugar analogues. They also showed a decrease in activity compared to compound **53** with the *n*-butyl substituent. From these data an *n*-butyl at N9 was chosen as a less polar substituent, although the ribose group itself (as in NBTI) led to much higher affinity. This became further apparent when synthesizing **54–56**, since in all cases a substantial reduction in affinity (16–577-fold) was observed when compared to the ribose-substituted analogues. This huge decrease in affinity had not been observed when comparing similar series of compounds on the actual transport in intact erythrocytes. In this typical assay the compounds need to enter the cells first, which is more easily achieved with the N9-butyl derivatives. Differences in activity were negligible in this case.[4]

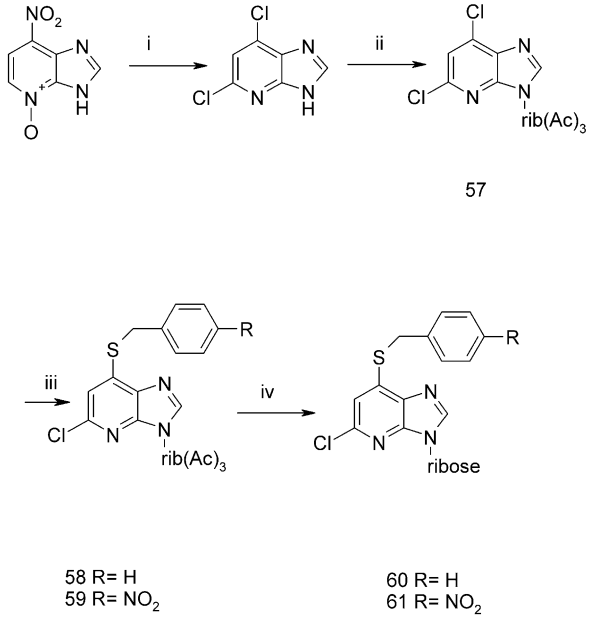


Scheme 8. Synthesis of N9-butylated compounds: (i) appropriate benzyl bromide, K_2CO_3, DMF, rt; (ii) NaH, BuBr, DMF, 18 h, rt.

Finally, in case of substitution of N1 with a carbon atom (1-deazapurines), the resulting nucleosides showed a decrease in affinity (**60** and **61** vs NBTI). Introduction of a nitro group in nucleoside **61** increased the affinity approx. tenfold relative to **60**.

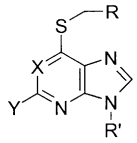
Conclusion

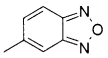
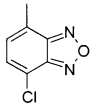
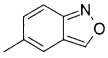
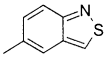
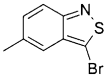
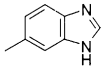
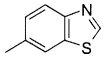
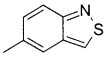
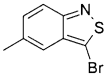
Inhibitors of nucleoside transport may have potential as drugs enhancing adenosine's actions in the CNS. As such their use may be in the treatment of chronic and/or neuropathic pain, epilepsy and other CNS-related disorders. Currently available ligands are often very



Scheme 9. Synthesis of 2-chloro-1-deaza analogues of NBTI: (i) $POCl_3$, DMF; (ii) $SnCl_4$, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose; (iii) 4-nitrobenzylmercaptane, Et_3N, DMF, rt, 18 h; (iv) NH_3/CH_3OH, 0 °C.

Table 1. Affinities of novel NBTI analogues for the nucleoside transport protein on human erythrocyte membranes



Compd	R	R′	X	Y	K_i (nM)
1	4-Nitrobenzyl	Ribose	N	H	0.59 (±0.07)
14		Ribose	N	H	1.5 (±0.1)
15		Ribose	N	H	9.7 (±0.2)
16		Ribose	N	H	25 (±2)
23		Ribose	N	H	96 (±1)
24		Ribose	N	H	666 (±47)
28		Ribose	N	H	108 (±2)
31		Ribose	N	H	42 (±4)
37		Ribose	N	H	191 (±12)
38		Ribose	N	H	42%[a]
41		Ribose	N	H	165 (±16)
43	4-Nitrobenzyl	THF	N	H	937 (±31)
44	4-Nitrobenzyl	THP	N	H	1200 (±100)
46	4-Nitrobenzyl	THP-*O*-C4	N	H	488 (±51)
47	4-Nitrobenzyl	Butan-4-ol	N	H	1140 (±40)
48	4-Nitrobenzyl	Cyclopentyl	N	H	736 (±67)
53	4-Nitrobenzyl	*n*-Butyl	N	H	238 (±38)
54		*n*-Butyl	N	H	5600 (±600)
55		*n*-Butyl	N	H	3100 (±200)
56		*n*-Butyl	N	H	682 (±12)
60	Benzyl	Ribose	C	Cl	670 (±31)
61	4-Nitrobenzyl	Ribose	C	Cl	47 (±4)

[a]Percentage of displacement at concentration of 10 μM.

hydrophilic, preventing substantial passage of the blood-brain barrier. In this study we addressed this issue by synthesizing and testing derivatives of NBTI (**1**) with decreased hydrophilicity through a focus on both C6- and N9-substitution. Many compounds proved to have affinities in the nanomolar range, although (slightly) less active than NBTI (**1**) itself. The substantial reduction in polarity achieved in some of the compounds is promising; they may have more favorable characteristics in aspects of absorption and distribution.

Experimental

Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 instrument. Samples were measured in $CDCl_3$, CD_3OD and/or DMSO-d_6, with Me_4Si as an internal standard; δ in ppm, *J* in Hz. Thin-layer chromatography (TLC) was performed by using plastic sheets precoated with silica gel 60 F254 (0.2 mm) type E (Merck). Chromatographic spots were visualized by UV light. Column chromatography was conducted on silica gel 60 (0.040–0.063 mm) unless otherwise noted. Melting points (uncorrected) were determined in open glass capillaries on an electrothermal apparatus. Mass spectra and accurate mass measurements were performed using a Finnigan MAT 900 spectrometer equipped with an electrospray interface. All commercial chemicals were used without further purification.

General procedure (A): coupling of the alkyl bromide with 6-mercaptopurine (riboside)

A mixture of 6-mercaptopurine riboside (1.0 equiv) or 6-mercaptopurine (1.0 equiv), anhydrous potassium carbonate (1.0 equiv), and 1.1 equiv of alkyl bromide in DMF was stirred at rt from 30 min up to 18 h. The mixture was poured in water and the solution was adjusted to pH = 7 with concentrated HCl. The mixture was extracted several times with ethyl acetate and the organic layer was dried ($MgSO_4$), filtered and evaporated. The product was purified by column chromatography eluting with EtOAc/MeOH, or by recrystallization.

General method (B): alkylation of the N9 position in purines by a Mitsunobu reaction

Diethyl azodicarboxylate (0.05 mL, 0.15 mmol) was added in portions to a mixture of 6-substituted purine (0.15 mmol), triphenyl phosphine (79 mg, 0.30 mmol) and the proper alcohol (0.30 mmol) in dry THF (2.7 mL). The mixture was stirred at 0 °C for 1 h, and then at rt for 10 h. After removal of the solvent in vacuo, the product was purified by flash column chromatography.

General method (C): N9-substitution of 6-mercaptopurine by a tetrahydropyranyl or tetrahydrofuranyl ring

To 60 mL of anhydrous ethyl acetate at 50 °C were added 6-(4-nitrobenzyl)mercaptopurine (1.0 equiv) and

p-toluene-sulfonic acid (0.1 equiv). The mixture was vigorously stirred and 2,3-dihydropyran or 2,3-dihydrofuran (3 equiv) added dropwise. The solution was stirred for 1 h and cooled to rt. Concentrated aqueous ammonia was added, the solution was stirred for 5 min, and washed twice with water. The ethyl acetate layer was dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization from petroleum ether (40–60 °C) afforded the product.

General method (D): coupling of the 6-substituted mercaptopurine with *n*-butyl bromide

6-Substituted mercaptopurine (1.0 equiv) was suspended in DMF. Sodium hydride (60% dispersion in mineral oil, 1.2 equiv) was added and the mixture was stirred at rt for 1 h under N_2 atmosphere. Then *n*-butyl bromide (1.0 equiv) was added and the stirring was continued at rt for 18 h under N_2 atmosphere. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was dried (K_2CO_3) and evaporated, to give a mixture of N9- and N7-butylated purines. The product was purified by column chromatography, while eluting with EtOAc/petroleum ether (40–60 °C). In all cases the first fraction contained the N9-substituted material.

6-(Benzo[1,2,5]oxadiazol-5-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (14). This compound was prepared by coupling 6-mercapto purine riboside with bromide **11**[7] by the general method **A**, in 46% yield. ^{1}H NMR (CD_3OD): 8.74 (s, 1H), 8.62 (s, 1H), 7.97 (s, 1H,), 7.82 (d, 1H, J = 9.51 Hz), 7.62 (dd, 1H, J = 8.04, 1.09 Hz), 6.08 (d, 1H, J = 5.85 Hz), 4.79 (s, 2H), 4.15 (m, 1H), 4.34 (m, 1H), 3.82 (m, 2H), 4.71 (m, 1H); ^{13}C NMR (CD_3OD): δ 160.8, 152.8, 149.4, 144.8, 144.1, 135.4, 117.4, 115.8, 90.9, 87.7, 75.8, 72.2, 63.0, 33.1; HR-MS calcd for $C_{17}H_{17}N_6O_5S$ m/z = 417.098 (M + H), found 417.1038.

6-(Benzo[1,2,5]oxadiazol-4-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (15). This compound was prepared by coupling the alkyl bromide **12**[7] with mercaptopurine riboside using general method **A**, in 78% yield. ^{1}H NMR (CD_3OD): δ 8.73 (s, 1H), 8.61 (s, 1H), 7.78 (d, 1H, J = 8.77 Hz), 7.65 (d, 1H, J = 6.58 Hz), 7.43 (dd, 1H, J = 8.78, 6.58 Hz), 6.08 (d, 1H, J = 5.85 Hz), 5.07 (s, 2H), 4.72 (m, 1H), 4.35 (dd, 1H, J = 5.12, 3.65 Hz), 4.16 (m, 1H), 3.82 (m, 2H); ^{13}C NMR (CD_3OD): δ 160.5, 156.9, 153.9, 152.6, 146.0, 136.3, 135.2, 133.4, 128.9, 117.4, 90.2, 88.1, 84.2, 76.2, 72.6, 63.6, 30.1. Mp 154–155 °C, decomp.; HR-MS calcd for $C_{17}H_{17}N_6O_5S$ m/z = 417.0981 (M + H), found 417.0035.

6-(6-Chloro-benzo[1,2,5]oxadiazol-4-ylmethyl-sulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (16). Coupling the alkyl bromide **13**[7] with mercaptopurine riboside by general method **A**, gave **16** in 70% yield. ^{1}H NMR (DMSO): δ 8.78 (s, 1H), 8.73 (s, 1H), 8.24 (d, 1H, J = 1.6 Hz), 7.67 (d, 1H, J = 1.6 Hz), 5.98 (d, 1H, J = 5.5 Hz), 5.51 (d, 1H, J = 5.9 Hz), 5.21 (d, 1H, J = 5.0 Hz), 5.08 (dd, 1H, J = 5.8 Hz), 5.05 (s, 2H), 4.57 (dd, 1H, J = 5.2, 5.5 Hz), 4.17 (d, 1H, J = 4.0 Hz), 3.95 (d, 1H, J = 4.0 Hz), 3.61 (m, 2H); ^{13}C NMR (CD_3OD): δ 177.4, 151.4, 149.3, 143.6, 137.2, 132.2, 128.9, 113.8, 95.4, 87.8, 85.7, 73.7, 70.2, 61.1, 27.4 and 11.9. Mp: 214–215 °C; HR-MS calcd for $C_{17}H_{16}N_6O_5SCl$ m/z = 451.0591 (M + H), found 451.0572.

6-(Benzo[*c*]isoxazol-5-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (23). Coupling of **21**[9] with mercaptopurine riboside was performed by general method **A**. The product was obtained in 54% yield. ^{1}H NMR (CD_3OD): δ 9.36 (s, 1H), 8.72 (s, 1H), 8.59 (s, 1H), 7.75 (s, 1H), 7.75 (d, 1H, J = 2.9 Hz), 7.51 (d, 1H, J = 2.9 Hz), 6.06 (d, 1H, J = 5.1 Hz), 4.70 (s, 2H), 4.35 (dd, 1H, J = 5.1, 5.9 Hz), 4.28 (m, 1H); 3.91 (m, 1H), 3.86 (m, 2H); ^{13}C NMR (CD_3OD): δ 156.9, 152.4, 144.4, 134.5, 130.2, 129.6, 120.0, 115.4, 113.1, 90.7, 87.5, 75.5, 71.2, 62.8, 33.6. Mp: 213–214 °C; HR-MS calcd for $C_{18}H_{18}N_5O_5S$ m/z = 416.1029 (M + H), found 416.1022.

6-(Benzo[*c*]isoxazol-7-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (24). Coupling of bromide **22**[9] with 6-mercaptopurine riboside was achieved by general method **A**. The product was obtained in 52% yield. ^{1}H NMR (CD_3OD): δ 9.47 (s, 1H), 8.74 (s, 1H), 8.58 (s, 1H), 7.58 (d, 1H, J = 8.8 Hz), 7.52 (d, 1H, J = 6.6 Hz), 6.99 (dd, 1H, J = 8.8, 6.6 Hz), 6.07 (d, 1H, J = 5.8 Hz), 4.96 (s, 2H), 4.71 (dd, 1H, J = J = 5.3 Hz), 4.32 (m, 1H); 4.14 (m, 1H); 3.89.72 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 157.8, 152.7, 144.6, 131.8, 125.7, 120.6, 90.9, 87.7, 75.7, 72.2, 63.1, 29.2. Mp: 200–201 °C; HR-MS calcd for $C_{18}H_{18}N_5O_5S_2$ m/z = 416.1029 (M + H), found 416.1024.

6-(Benzo[*c*]isothiazol-5-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (28). This compound was prepared from bromide **27**[10] (187 mg, 0.82 mmol) and 6-mercaptopurine riboside (214 mg, 0.75 mmol) by general method **A**. The solvent was decanted from the white precipitate and after washing with water and drying over silica blue in vacuo **28** (81 mg, 25%) was obtained as an off white solid. ^{1}H NMR (DMSO-d_6): δ 9.73 (s, 1H), 8.84 (s, 1H), 8.75 (s, 1H), 8.00 (s, 1H), 7.78 (d, 1H, J = 9.5 Hz), 7.59 (d, 1H, J = 9.5 Hz), 6.03 (d, 1H, J = 5.1 Hz), 5.30 (broad, 3H, 3 × OH), 4.82 (s, 2H), 4.62 (dd, 1H, J = 5.1, 5.9 Hz), 4.20 (m, 1H); 3.99 (m, 1H), 3.66 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 160.4, 158.9, 151.5, 148.3, 147.0, 143.4, 134.1, 133.6, 131.1, 130.6, 121.8, 121.3, 87.8, 85.7, 74.0, 70.2, 61.2, 31.6; Mp: 183–186 °C; HR-MS calc. for $C_{18}H_{18}N_5O_4S_2$ m/z = 432.0800 (M + H), found 432.0835.

3-Bromo-5-methyl-2,1-benzisothiazole (29). To a solution of compound **26**[10] (1.49 g, 10.0 mmol) in dry THF (50 mL) at −80 °C was added *n*-BuLi (11.2 mmol, 7.0 mL of a 1.6 M solution in *n*-hexane) dropwise. The black solution was stirred at −75 °C for 20 min after which bromine (1.0 mL, 19.9 mmol) was added. The mixture was slowly warmed to rt and poured into a 1 N HCl (100 mL) solution. Extraction with diethyl ether (3 × 50 mL), drying ($MgSO_4$), evaporation of the solvent and purification by silicagel column chromatography [petroleum ether (40–60)/EtOAc = 95/5] gave **29** (1.55 g, 68%) as a red oil. ^{1}H NMR ($CDCl_3$): δ 7.66 (d, 1H, J = 9.5 Hz), 7.33 (d, 1H, J = 9.5 Hz), 7.30 (s, 1H), 2.46 (s,

3H); ^{13}C NMR ($CDCl_3$): δ 160.1, 134.6, 134.1, 131.8, 130.0, 121.2, 118.0, 21.3.

3-Bromo-5-bromomethyl-2,1-benzisothiazole (30). This compound was prepared as **11** by benzylic bromination in the presence of *N*-bromosuccinimide[7] in 84% yield. ^{1}H NMR ($CDCl_3$): δ 4.61 (s, 2H); 7.49 (d, 1H, J = 9.5 Hz); 7.61 (s, 1H); 7.78 (d, 1H, J = 9.5 Hz); ^{13}C NMR ($CDCl_3$): δ 160.9, 134.8, 134.0, 133.2, 130.7, 122.9, 120.0, 33.2.

6-(3-Bromo-benzo[*c*]isothiazol-5-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (31). This compound was prepared from bromide **30** (169 mg, 0.55 mmol) and 6-mercaptopurine riboside (141 mg, 0.50 mmol) by general method **A** in 86% yield as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.85 (s, 1H), 8.76 (s, 1H), 7.67 (d, 1H, J = 9.5 Hz); 7.79 (m, 2H), 6.03 (d, 1H, J = 5.5 Hz), 5.34 (b, m, 3H), 4.87 (s, 2H), 4.62 (dd, 1H, J = 5.1, 5.5 Hz), 4.21 (m, 1H), 4.00 (m, 1H), 3.69 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 160.4, 158.7, 151.5, 148.4, 143.5, 135.7, 133.8, 132.8, 131.6, 131.1, 122.0, 119.6, 87.9, 85.7, 73.8, 70.2, 61.2, 31.3; Mp: 129–131 °C; HR-MS calcd for $C_{18}H_{17}BrN_5O_4S_2$ m/z = 509.9905 (M + H), found 509.9918.

(Benzimidazol-5-yl)-methanol (34). To a suspension of $LiAlH_4$ (2.4 g, 63 mmol) in dry THF (73 mL) was added dropwise a solution of ester **32**[13] (4.0 g, 21.05 mmol) in dry THF (22 mL). The mixture was refluxed, the excess of $LiAlH_4$ destroyed cautiously by a saturated solution of NH_4Cl. The organic layer was separated and the water layer was extracted with ethyl acetate. The combined organic layers were dried (K_2CO_3) and evaporated to give **34** as a yellow oil that crystallised spontaneously (yield 70%). ^{1}H NMR (CD_3OD): δ 8.13 (s, 1H), 7.61 (s, 1H), 7.57 (d, 1H, J = 8.04 Hz), 7.26 (d, 1H, J = 8.77 Hz), 5.07 (s, 2H).

(Benzimidazol-4-yl)-methanol (35). The same reduction reaction as for ester **32** was performed on ester **33**; in this case the yield was 88%. ^{1}H NMR (CD_3OD): δ 8.15 (s, 1H), 7.53 (m, 1H), 7.24 (m, 2H), 4.98 (s, 2H).

6-(1*H*-Benzimidazol-5-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (37). (Cyanomethyl)-trimethyl-phosphonium iodide **36** (299 mg, 1.23 mmol) was added to a mixture of alcohol **35** (200 mg, 1.35 mmol), 6-mercaptopurine riboside (284 mg, 1.00 mmol), and DIPEA (0.25 mL) in propionitrile (3 mL). The mixture was heated at 90 °C and some drops of DMF were added to obtain a clear solution. The mixture was stirred overnight at 90 °C, cooled to rt and water was added. Upon standing at rt, **37** precipitated. **37** was obtained by collection on a filter and drying in vacuo (65% yield). ^{1}H NMR (DMSO-d_6): δ 8.83 (s, 1H), 8.64 (s, 1H), 8.21 (broad s, 1H), 7.79 (s, 1H), 7.59 (d, 1H, J = 8.0 Hz), 7.41 (d, 1H, J = 8.0 Hz), 6.10 (d, 1H, J = 5.7 Hz), 4.85 (s, 2H), 4.72 (dd, 1H, J = 5.4, 5.0 Hz), 4.35 (dd, 1H, J = 5.4, 5.0 Hz), 4.25 (d, 1H, J = 5.0 Hz), 3.81 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 162.0, 152.8, 149.5, 144.5, 133.7, 132.6, 125.2, 90.5, 87.6, 75.7, 72.2, 63.0, 33.9. Mp: 196–198 °C; HR-MS calcd for $C_{18}H_{19}N_6O_4S$ m/z = 415.1200 (M + H), found 415.1155.

6-(1*H*-Benzimidazol-4-ylmethylsulfanyl)-9-β-D-ribofuranosyl-9*H*-purine (38). Starting from alcohol **35** and applying the same reaction conditions as for **37** gave the coupled product **38** in 44% yield. ^{1}H NMR (CD_3OD): δ 8.76, 8.58 (2 × s, 2H), 8.20 (s, 1H), 7.54 (m, 1H), 7.41 (d, 1H, J = 7.31 Hz), 7.20 (m, 1H), 6.08 (d, 1H, J = 5.84 Hz), 5.03 (s, 2H), 4.72 (m, 1H), 4.35 (m, 1H), 4.15 (m, 2H), 3.83 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 162.4, 152.7, 149.1, 144.5, 143.2, 142.6, 132.5, 124.6, 124.2, 123.8, 90.0, 87.7, 75.7, 72.2, 63.1, 61.5, 29.8. Mp = decomp. 136–138 °C; m/z = 415.1200 (M + H), found 415.1123.

(Benzothiazol-6-yl)-methanol (40). To a suspension of $LiAlH_4$ (76 mg, 2.0 mmol) in dry THF (20 mL) under N_2 was added dropwise and at 0 °C a solution of ester **39** (207 mg, 1.0 mmol) in dry THF (6.7 mL). After 2 h, the ice bath was removed and the mixture was stirred overnight at rt. After cooling to 0 °C the excess of $LiAlH_4$ was destroyed very cautiously with a saturated solution of NH_4Cl. The organic layer was removed and the aqueous layer was extracted with EtOAc. The combined organic layers were dried (Na_2CO_3) and evaporated, affording an orange oil that was purified by column chromatography, eluting with EtOAc/MeOH (90/10). ^{1}H NMR (CD_3OD): δ 9.20 (s, 1H), 8.02 (m, 2H), 7.53 (d, 1H, J = 8.04 Hz), 4.76 (s, 2H).

6-(Benzothiazol-6-ylmethylsulfanyl)-9-(β-D-ribofuranosyl)-purine (41). (Cyanomethyl)trimethyl-phosphonium iodide (45 mg) was added to a mixture of **40**[14] (33 mg, 0.2 mmol), 6-mercaptopurine riboside (42 mg, 0.2 mmol) and DIPEA (0.04 mL) in propionitrile (0.43 mL). The mixture was heated at 90 °C and a few drops of DMF were added to obtain a clear solution. Stirring was continued overnight at 90 °C. The mixture was cooled to rt, water was added and slowly a precipitate was formed. The solid was collected and dried in vacuo. ^{1}H NMR (CD_3OD): δ 9.20 (s, 1H), 8.74 (s, 1H), 8.59 (s, 1H), 8.18 (s, 1H), 7.98 (d, 1H, J = 8.78 Hz), 7.67 (d, 1H, J = 8.77 Hz), 6.07 (d, 1H, J = 5.11 Hz), 4.84 (s, 2H), 4.72 (m, 1H), 4.35 (m, 1H), 4.17 (m, 2H), 3.83 (m, 1H); ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 161.5, 157.1, 153.5, 152.8, 149.5, 144.6, 137.4, 135.2, 129.0, 124.0, 123.7, 90.6, 87.6, 75.7, 72.2, 63.0, 33.2; Mp = decomp. 140 °C. HR-MS calcd for $C_{18}H_{18}N_5O_4S_2$ m/z = 432.5100 (M + H), found 432.4787.

6-(4-Nitrobenzylsulfanyl)-9-(tetrahydro-furan-2-yl)-9*H*-purine (43). This compound was prepared from **42** by general method **C** in 67% yield. ^{1}H NMR ($CDCl_3$): δ 8.75 (s, 1H), 8.15 (s, 1H), 7.62 (d, 1H, J = 8.9 Hz), 5.52 (d, 1H, J = 8.9 Hz), 4.75 (m, 1H), 4.44 (m, 2H), 2.19 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 158.6, 151.2, 148.5, 145.9, 141.2, 129.9, 128.6, 127.9, 126.5, 123.1, 121.1, 97.1, 64.8, 61.3, 56.1, 32.1, 23.2; HR-MS calcd for $C_{16}H_{16}N_5O_3S$ m/z = 358.3919 (M + H), found 358.3911.

6-(4-Nitrobenzylsulfanyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (44). This compound was obtained from **42** by general method **A** in 62% yield. ^{1}H NMR ($CDCl_3$): δ 8.74 (s, 1H), 8.18 (d, 1H, J = 9.2 Hz), 8.13 (s, 1H), 7.65 (d, 1H, J = 9.2 Hz), 5.74 (m, 1H), 4.72 (s, 2H), 4.15 (m,

2H), 3.78 (m, 3H), 2.29 (m, 3H), 1.75 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 158.6, 151.1, 148.4, 145.6, 141.2, 129.7, 128.1, 127.0, 126.7, 123.4, 121.5, 97.9, 64.6, 61.8, 36.1, 32.3, 31.4, 23.6; HR-MS calcd for $C_{17}H_{18}N_5O_3S$ $m/z = 372.1131$ (M + H), found 372.1104.

4-[6-(4-Nitrobenzylsulfanyl)-9*H*-purine-9-yl]butan-1-ol (47). Compound **46** was made from **42** by general method **B** in 86% yield. ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1H), 8.05 (d, 1H, $J = 9.2$ Hz), 7.97 (s, 1H), 7.70 (d, 1H, $J = 9.2$ Hz), 4.65 (s, 2H), 4.42 (m, 1H), 4.21 (m, 3H), 3.72 (m, 3H), 3.33 (m, 3H), 2.12 (m, 3H), 1.51 (m, 4H).

Deprotection of **46** was achieved with *p*-toluene sulfonic acid to give **47** in 65% yield. ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1H), 8.36 (s, 1H), 8.10 (d, 2H, $J = 9.2$ Hz), 7.70 (d, 2H, $J = 9.2$ Hz), 4.82 (broad s, 1H), 4.74 (s, 2H), 4.43 (t, 2H, $J = 7.3$ Hz), 3.57 (t, 2H, $J = 7.3$ Hz), 1.97 (m, 2H), 1.51 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 159.2, 151.7, 148.9, 147.2, 145.8, 143.0, 132.1, 131.9, 129.9, 128.6, 123.7, 62.1, 43.9, 31.8, 29.2, 27.0. Mp: 128–130 °C; HR-MS calcd for $C_{16}H_{19}N_5O_3S$ $m/z = 360.1130$ (M + H), found 360.1123.

6-(4-Nitrobenzylsulfanyl)-9-cyclopentyl-9*H*-purine (48). This compound was obtained from **42** by method **B** in 56% yield. ^{1}H NMR (CD_3OD): δ 8.73 (s, 1H), 8.16 (d, 2H, $J = 9.2$ Hz), 8.02 (s, 1H), 7.65 (d, 2H, $J = 9.2$ Hz), 4.96 (m, 1H), 4.72 (s, 2H), 2.29 (m, 1H), 1.97.80 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 158.6, 151.1, 148.4, 145.6, 141.2, 129.7, 126.7, 123.4, 56.1, 32.3, 31.0, 23.6. Mp: 92–94 °C; HR-MS calcd for $C_{17}H_{18}N_5O_2S$ $m/z = 356.1181$ (M + H), found 356.1179.

9-But-1-yl-6-(4-nitrobenzylsulfanyl)-9*H*-purine (53). This compound was prepared by general method **A** from **42** in 98% yield. ^{1}H NMR (DMSO-d_6): δ 8.76 (s, 1H), 8.52 (s, 1H), 8.18 (d, $J = 8.77$ Hz), 7.76 (d, $J = 8.77$ Hz), 4.79 (s, 2H), 4.25 (t, 2H, $J = 7.31$ Hz), 1.82 (tt, 2H, $J = 7.31$ Hz), 1.24 (tq, 2H, $J = 7.31$ Hz), 0.89 (t, 3H, $J = 7.31$ Hz); HR-MS calcd for $C_{16}H_{18}N_5O_2S$ $m/z = 344.1181$ (M + H), found 344.1148.

6-(Benzo[1,2,5]oxadiazol-4-ylmethylsulfanyl)-9*H*-purine (50). This compound was prepared by general method **A**, from **49** and bromide **12** in 88% yield. ^{1}H NMR (CD_3OD): δ 8.77 (s, 1H), 8.13 (s, 1H), 7.72 (d, 1H, $J = 8.77$ Hz), 7.57 (d, 1H, $J = 6.58$ Hz), 7.32 (dd, 1H, $J = 8.77$, 5.84 Hz), 5.07 (s, 2H).

1-[6-(Benzo[1,2,5]oxadiazol-4-ylmethylsulfanyl-9*H*-purine-9-yl]butane (54). This compound was made by general method **D** from **50** and *n*-butyl bromide in 63% yield. ^{1}H NMR (CD_3OD): δ 8.76 (s, 1H), 7.93 (s, 1H), 7.63 (d, 1H, $J = 8.77$ Hz), 7.52 (d, 1H, $J = 6.58$ Hz), 7.26 (dd, 1H, $J = 6.58$, 8.78 Hz), 4.97 (s, 2H), 4.20 (t, 2H, $J = 7.31$ Hz), 1.83 (t, 2H, $J = 7.31$ Hz), 1.28 (t, 2H, $J = 7.31$ Hz), 0.89 (t, 3H, $J = 7.31$ Hz); ^{13}C NMR ($CDCl_3$): δ 151.5, 145.6, 142.7, 129.8, 123.4, 121.3, 98.1, 43.6, 31.7, 31.4, 19.6, 13.2; HR-MS calcd for $C_{16}H_{17}N_6OS$ (M + H), 341.1106 found 341.1184.

1-[6-(Benzo-[2,1]-isothiazol-5-yl)methylsulfanyl-9*H*-purine-9-yl]butane (55). This compound was made by stirring a mixture of **51** (315 mg, 1.38 mmol), 6-mercaptopurine (245 mg, 1.55 mmol) and K_2CO_3 (228 mg, 1.65 mmol) in DMF (5 mL) at rt for 3 h. (general method **A**). After addition of water (30 mL) the mixture was allowed to stand at 5 °C for 4 days. An oily residue separated from the water layer. After removal of the water layer, toluene (20 mL) was added and evaporated in vacuo. This procedure was repeated twice after which the remaining residue was dissolved in DMF (10 mL). At 0 °C NaH (60% dispersion in mineral oil, 70 mg, 1.75 mmol) was added. After 30 min *n*-butylbromide (200 μL, 1.85 mmol) was added. The reaction was stirred overnight at rt and poured into water (40 mL). Extraction with EtOAc (3 × 20 mL), drying ($MgSO_4$) and concentration in vacuo gave an orange oil that was purified by silicagel column chromatography (*n*-heptane/EtOAc = 2/1) to afford **55** (245 mg, 50%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 9.11 (s, 1H), 8.77 (s, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.79 (d, 1H, $J = 9.5$ Hz), 7.55 (d, 1H, $J = 9.5$ Hz), 4.77 (s, 2H), 4.28 (t, 2H, $J = 6.6$ Hz), 1.89 (m, 2H), 1.33 (m, 2H), 0.95 (t, 3H, $J = 7.3$ Hz); ^{13}C NMR ($CDCl_3$): δ 160.6, 159.5, 151.4, 148.4, 143.9, 142.6, 134.2, 133.5, 130.9, 130.3, 121.4, 121.2, 43.4, 32.3, 31.6, 19.5, 13.1. Mp: 93–94 °C; HR-MS calcd for $C_{17}H_{17}N_5BrS_2$ $m/z = 434.0109$ (M + H), found 434.0080.

6-(3-Bromo-benzo-[2,1]-isothiazol-5-yl)methylsulfanyl-9*H*-purine (52). This compound was made by general method **A**, from bromide **30** in 90% yield as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.79 (s, 1H), 8.48 (s, 1H), 7.79 (m, 2H), 7.67 (d, 1H, $J = 9.5$ Hz), 4.85 (s, 2H), 3.33 (b, s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 160.3, 156.9, 151.3, 150.6, 143.7, 135.9, 133.8, 132.7, 131.7, 129.2, 122.0, 119.5, 31.3.

1-[6-(3-Bromo-benzo[*c*]isothiazol-5-yl)methylsulfanyl-9*H*-purine-9-yl]butane (56). This compound was prepared by the general method **D** from **52** and *n*-butylbromide in 69% yield. ^{1}H NMR ($CDCl_3$): δ 8.78 (s, 1H), 7.95 (s, 1H), 7.71 (m, 2H), 7.57 (d, 1H, $J = 9.5$ Hz), 4.78 (s, 2H), 4.25 (t, 2H, $J = 6.6$ Hz), 1.89 (m, 2H), 1.34 (m, 2H), 0.96 (t, 3H, $J = 7.3$ Hz); ^{13}C NMR ($CDCl_3$): δ 160.7, 159.3, 151.4, 148.5, 142.6, 134.9, 134.0, 131.9, 131.1, 130.9, 122.1, 119.7, 43.5, 32.3, 31.7, 19.6, 13.2. Mp: 118–119 °C; HR-MS calcd for $C_{17}H_{18}N_5S_2$ $m/z = 356.1003$ (M + H), found 356.0927.

5-Chloro-7-benzylsulfanyl-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (60). A mixture of **57** (166 mg, 0.37 mmol), benzylthiol (65 mg, 0.60 mmol) and 0.02 mL of Et_3N in 2 mL of DMF was stirred at rt for 18 h under N_2 atmosphere. The solution was extracted with ether, dried and purified by flash chromatography (PE/EA, 1:1), yielding 5-chloro-7-(benzylsulfanyl)-3-(2′,3′,5′-tri-*O*-acetyl -β-D-ribofuranosyl)-3H-imidazo[4,5-*b*]pyridine **58** (168 mg) in 85% yield. ^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H), 7.42.31 (m, 5H), 7.08 (s, 1H), 6.23 (d, 1H, $J = 5.1$ Hz), 5.85 (dd, 1H, $J = 5.1$, 5.9 Hz), 5.65 (dd, 1H, $J = 5.1$, 5.9 Hz), 4.43 (s, 2H), 4.38 (m, 2H), 2.28 (s, 1H), 2.12 (s, 1H), 1.90 (s, 1H).

Deprotection of **58** with a saturated solution of ammonia in methanol at 0 °C for 18 h gave 83 mg of **60** (65%). ^{1}H NMR (CD_3OD): δ 8.60 (s, 1H), 7.40.29 (m, 5H),

7.25 (s, 1H), 6.04 (d, 1H, $J = 5.1$ Hz), 4.69 (dd, 1H, $J = 5.1$, 5.9 Hz), 4.43 (s, 2H), 4.36 (dd, 1H, $J = 5.1$, 5.9 Hz), 3.94.80 (m, 2H); ^{13}C NMR (CD_3OD): δ 147.2, 145.8, 145.1, 144.3, 137.2, 130.0, 129.8, 128.5, 126.8, 115.2, 90.7, 87.2, 75.2, 71.8, 62.8, 35.7. Mp: 154–156 °C; HR-MS calcd for $C_{18}H_{19}N_3O_4SCl$ $m/z = 408.0785$ (M + H), found 408.0788.

5-Chloro-7-(4-nitro-benzylsulfanyl)-3-β-D-ribofurano-syl-3*H*-imidazo[4,5-*b*]pyridine (61). A mixture of **57** (75 mg, 0.17 mmol), *p*-nitro-benzylthiol (85 mg, 0.50 mmol) and 0.1 mL of Et_3N in 5 mL of DMF was stirred at rt for 18 h under N_2 atmosphere. The solution was extracted with ether, dried and purified by flash chromatography (PE/EA. 1:1), yielding 72 mg of 5-chloro-7-(4-nitroben-zylsulfanyl)-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofurano-syl)-3H-imidazo[4,5-*b*]pyridine **59** (75%). ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 2H, $J = 8.0$ Hz), 8.15(s, 1H), 7.58 (d, 2H, $J = 8.0$ Hz), 7.04 (s, 1H), 6.21 (d, 1H, $J = 5.1$ Hz), 5.83 (dd, 1H, $J = 5.1$, 5.9 Hz), 5.64 (dd, 1H, $J = 5.1$, 5.9 Hz), 4.63 (s, 2H), 4.38 (m, 2H), 2.14 (s, 1H), 2.12 (s, 1H), 2.08 (s, 1H). Deprotection of **59** with saturated solution of ammonia in methanol at 0 °C for 18 h followed by evaporation and column chromatography ($MeOH/CH_2Cl_2 = 85/15$) gave 50 mg of **61** (88%). ^{1}H NMR ($CDCl_3$): δ 8.57 (s, 1H), 8.14 (d, 2H, $J = 8.0$ Hz), 7.66 (d, 2H, $J = 8.0$ Hz), 7.18(s, 1H), 6.03 (d, 1H, $J = 5.1$ Hz), 4.89 (s, 2H), 4.69 (dd, 1H, $J = 5.1$, 5.9 Hz), 4.43 (m 1H), 3.88.78 (m, 2H). ^{13}C NMR (CD_3OD): 147.2, 145.8, 145.1, 144.3, 137.2, 133.3, 130.0, 129.8, 128.8, 115.5, 90.9, 87.5, 75.5, 72.2, 63.1, 36.1. Mp: 162–165 °C; HR-MS calcd for $C_{18}H_{18}N_4O_6ClS$ $m/z = 453.0635$ (M + H), found 453.0614.

Erythrocytes and membrane preparation. Whole human blood (Blood Bank, Leiden University Medical Center) was stirred in lysis buffer (1/2 v/v, 10 mM $MgCl_2$ in 10 mM Tris–HCl, pH 8.0 at 25 °C) for 1 h. After homogenization it was centrifuged for 50 min at 19,000 rpm. The supernatant was removed and the pellet was dissolved in ice-cold water and centrifuged again for 50 min. This procedure was repeated two more times. After removal of the last supernatant 25 mL of buffer (50 mM Tris–HCl, pH 7.4 at 25 °C) was added to the final pink pellet. This suspension was homogenized and the ghosts were collected. Aliquots were stored at −80 °C until further use.

[^{3}H]NBTI binding assay. Saturation and displacement equilibrium NBTI binding to membranes prepared from human erythrocytes (ghosts) was determined at 25 °C based on a method previously described.[3]

Acknowledgements

This work was financially supported by Grünenthal GmbH.

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