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A broad spectrum anticancer nucleoside with selective toxicity against human colon cells in vitro

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ABSTRACT

2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[*N*-(methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)-adenosine, a new member of the N^6,5′-bis-ureidoadenosine class of anticancer nucleosides, is found to exhibit broad spectrum antiproliferative activity. A majority of the cell lines in the NCI-60 are inhibited with an average GI_{50} = 3.13 μM. Selective toxicity against human colon cancer cell lines (COLO 205, HCC-2998, HCT-116, HT29, KM12) was also exhibited (LC_{50}'s = 6–10 μM).

Recently, we reported the synthesis and biological evaluation of a new class of antiproliferative bis-ureidoadenosine derivatives typified by compounds **1**–**3** (Fig. 1).[1] A preliminary SAR study revealed that substitution in the NE, NW, and SE quadrants of these molecules was essential for optimal (low μM) antiproliferative activities (Fig. 2). Left in doubt from these preliminary studies was the essential character of the 3′-C functionalization found in the SW quadrant of compounds **1** and **2**. A single-dose screen of compound **3** suggested that the 3′-C carboxymethyl moieties of **1** and **2** might not be essential for activity in this class of compounds. Here, we report the multi-dose data from the NCI-60 screen of compound **3**, which confirms that substitution at the 3′-position with the unnatural (and synthetically challenging) 3′-C carboxymethyl moieties of **1** and **2** is not necessary for retention of antiproliferative activity of the parent compounds (Table 1).[2] Indeed, compound **3** generally exhibited greater potency than either compounds **1** or **2**, and also exhibited selective toxicity toward several of the cells in the NCI-60 panel (Table 2). In contrast, compounds **1** and **2** were not as potent and did not exhibit any significant degree of selective toxicity (Table 2). With only a few exceptions, LC_{50} values for compounds **1** and **2** were >100 μM, and no clear trend for selective toxicity was apparent. Compound **3** on the other hand had LC_{50}'s in the 6–10 μM range for five of the six human colon cancer cell lines, and three of the renal cell lines exhibited similar levels of toxicity. The average GI_{50} for compound **3** was 3.13 μM, compared to 7.57 and 17.2 μM for compounds **1** and **2**, respectively (Table 1).[3] Thus compound **3** exhibited both greater potency and selective toxicity than either lead compounds **1** or **2**.

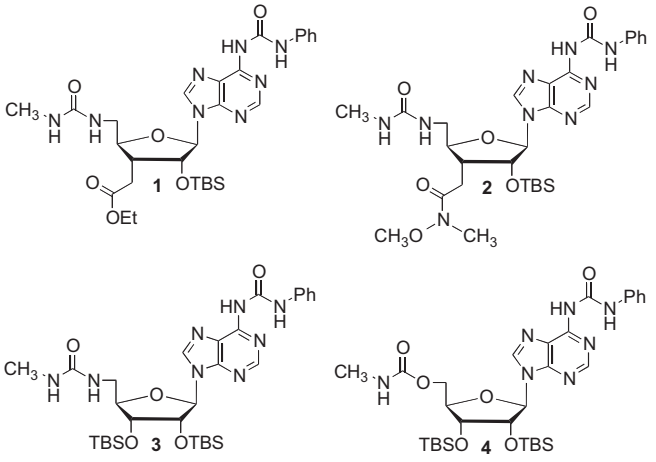

Figure 1.

The synthesis of compound **3** required six steps from commercially available adenosine (Scheme 1).[1] In an effort to reduce the number of steps and to test the antiproliferative activity of the isosteric, yet synthetically more tractable, 5′-*N*-methylcarbamoyl derivative, compound **4** was also prepared and tested. Its synthesis required four steps and gave compound **4** in 61% overall yield from adenosine.[4]

Unfortunately, and perhaps not too surprisingly given that compound **4** has one less potential hydrogen bond donor relative to compound **3**, the single-dose NCI-60 screening data indicated that compound **4** had substantially inferior antiproliferative activity in the single-dose growth inhibition assay (Table 1).

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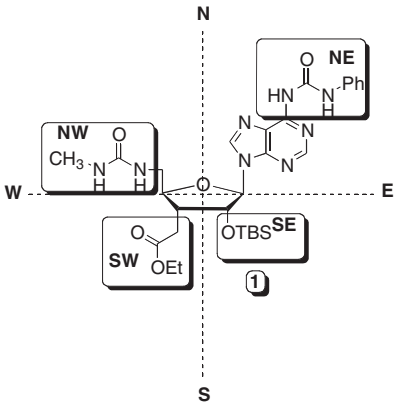


Figure 2.

Although differences in pharmacological and/or biological parameters associated with the different cell lines could be important, the difference in antiproliferative activities between **3** and **4** could be at least partially attributable to the loss of favorable hydrogen bonding in compound **4** relative to compound **3** and their (assumed) mutual biological receptor(s). A classic example of the effect of an NH to O substitution is seen in the development of Vancomycin resistance when D-alanine (NH) is substituted by D-lactate (O) in the peptidoglycan cell-wall precursor of Gram-positive bacteria.[5] The NH to O substitution in the L-lys-D-ala-D-ala to L-lys-D-ala-D-lac mutation causes a loss of binding affinity of at least 1000-fold between Vancomycin and its wild-type ligand. In a similar, but less profound manner, loss of an NH moiety in compound **4** relative to **3** could contribute to decreased binding between **4** and its biological receptor. Another factor that could influence decreased binding of **4** is the loss of conformational rigidity resulting from the absence of an intramolecular hydrogen bond between N^3 and the 5′-NH of the *N*-methylureido moiety. Such loss of conformational rigidity would be expressed as an increased *syn*/*anti* glycosyl fluctionality that could lead to decreased binding of compound **4** relative to the more conformationally rigid compound **3**.[6]

Intramolecular hydrogen bonds between N^3 and 5′-hydrogen-bearing substituents have been reported to impart significant conformational rigidity to adenosine derivatives that otherwise exist in dynamic *syn*/*anti* glycosyl equilibria.[7] In one such notable case, intramolecular hydrogen bonding between the 5′-OH and N^3 of 1-deaza-3′-*O*-methyladenosine locks the nucleoside in the *syn* conformation in the solid state, and ^{1}H–^{1}H-NOE difference spectra suggested a high population of the *syn*-conformer in solution.[8] In the case of compound **3**, NOESY and $^3J(^1\text{H}–^1\text{H})$ coupling data suggest that compound **3** exists primarily in the *syn* conformation in solution. In contrast, these data suggest that compound **4** is much more conformationally labile (Fig. 3). It has been well documented that for purine nucleosides, a correlation exists between the *syn*/*anti* equilibrium about the glycosidic bond and the conformational bias (C2′-*endo* vs C3′-*endo*) of the sugar. The C2′-*endo* (S) conformation correlates with a predominant *syn* glycosyl conformation (χ = 10–30°), whereas the C3′-*endo* (N) conformation correlates with the *anti* glycosyl conformation (χ = 200°–210°).[7] To a first approximation, the equilibrium constant (K_{eq}) can be calculated from the observed $J_{1',2'}$ and $J_{3',4'}$ according to Eq. 1, where X_S and X_N correspond to the mole fraction of S conformer and N conformer, respectively. Equilibrium constants calculated with this expression compare very favorably to those obtained by a full pseudorotational analysis.[7,9]

$$(K_{eq}) = X_S/X_N = J_{1',2'}/J_{3',4'} \quad (1)$$

Table 1
Results of growth inhibition assays (GI_{50}, μM)[a] (growth percent)[b]

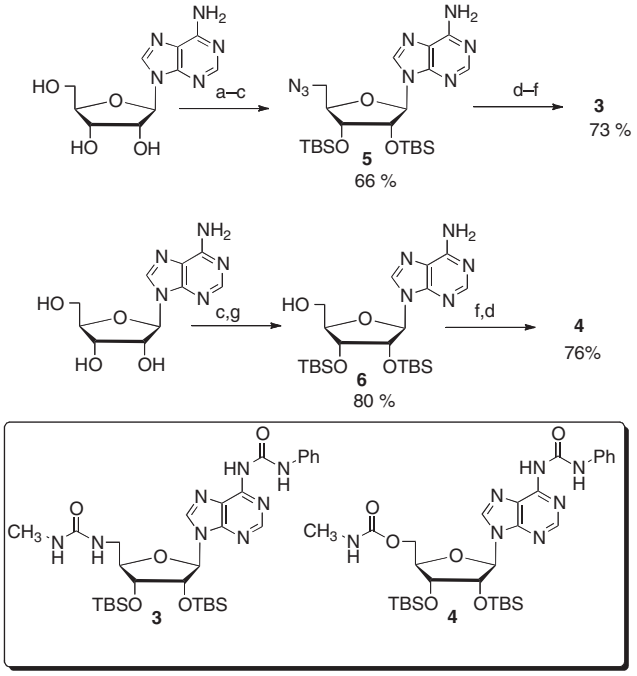
	1[a]	**2**[a]	**3**[a]	**3**[b]	**4**[b]
Leukemia					
CCRF-CEM	6.69	6.37	3.59	43	—
HL-60(TB)	3.01	1.81	—	24	113
K-562	3.59	3.12	—	34	75
MOLT-4	2.39	2.13	—	24	75
RPMI-8226	1.09	1.58	3.27	20	88
Non-small cell lung cancer					
A549/ATCC	4.18	9.35	2.90	23	81
EKVX	17.7	26.4	3.70	23	104
HOP-62	8.96	24.9	4.98	62	104
HOP-92	< 0.01	2.71	1.56	−3	55
NCI-H226	>100	41.9	3.10	49	93
NCI-H23	33.3	57.2	3.17	36	98
NCI-H322M	>100	>100	6.86	87	118
NCI-H460	5.54	7.49	1.72	−7	103
NCI-H522	4.36	11.1	2.39	46	53
Colon cancer					
COLO 205	3.84	12.3	1.89	−100	106
HCC-2998	>100	30.6	2.02	−1	—
HCT-116	3.20	4.20	2.11	0	67
HCT-15	8.50	6.47	3.62	21	103
HT29	4.20	5.37	2.02	−68	81
KM12	3.95	23.9	2.13	−4	110
SW620	4.80	>100	—	36	98
Melanoma					
LOX IMVI	5.46	7.30	1.82	13	87
MALME-3M	10.3	11.4	2.98	49	114
M14	2.51	15.2	4.33	33	102
SK-MEL-2	5.42	14.9	2.45	57	83
SK-MEL-28	6.85	7.77	—	60	102
SK-MEL-5	4.34	5.81	1.98	48	101
UACC-257	5.68	22.6	3.78	43	—
UACC-62	>100	41.9	3.98	42	106
CNS cancer					
SF-268	6.53	8.29	3.58	36	85
SF-295	5.73	9.09	3.84	45	108
SF-539	5.19	22.3	3.07	33	97
SNB-19	29.0	>100	4.57	76	114
SNB-75	4.56	12.7	2.43	46	77
U251	4.69	5.66	2.96	17	86
Ovarian cancer					
IGROV1	3.85	3.72	2.74	53	111
OVCAR-3	4.59	7.11	2.60	−29	107
OVCAR-4	12.3	53.0	3.44	30	86
OVCAR-5	31.1	38.2	5.24	69	120
OVCAR-8	4.92	9.02	4.01	33	89
SK-OV-3	21.0	52.7	6.88	78	106
Renal cancer					
786-0	2.00	9.01	2.04	17	95
A498	3.34	3.87	2.03	—	98
ACHN	8.55	14.4	3.83	21	95
CAKI-1	29.7	53.8	2.70	60	106
RXF393	2.01	9.74	2.14	−37	94
SN12C	9.10	85.3	1.81	−79	101
TK-10	12.4	20.6	4.29	34	96
UO-31	12.1	7.79	2.50	48	106
Breast cancer					
BT-549	>100	29.0	2.94	40	—
HS578T	3.60	5.79	—	61	98
MCF7	3.42	5.59	2.69	0	88
MDA-MB-231/ATCC	3.96	12.3	2.61	38	110
MDA-MB-468	—	—	3.35	36	104
T-47D	2.55	13.9	2.87	14	116
Prostate cancer					
DU-145	4.97	16.6	2.58	52	106
PC-3	2.25	—	2.69	8	59

[a] Multi-dose growth inhibition, GI_{50} determined from dose–response curve.
[b] Single-dose growth inhibition percent calculated as: $[(T_i - T_z)/(C - T_z)] \times 100$ for $T_i \geqslant T_z$; $[(T_i - T_z)/T_z)] \times 100$ for $T_i < T_z$; where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h (10 μM test compound); C = absorbance of control at t = 48 h.

Table 2
Results of multi dose growth inhibition assay (LC_{50}, μM)[a]

Cell line	1	2	3
Leukemia			
CCRF-CEM	>100	>100	>100
HL-60(TB)	>100	>100	—
K-562	>100	>100	—
MOLT-4	>100	>100	—
RPMI-8226	59	>100	>100
Non-small cell lung cancer			
A549/ATCC	79.1	>100	>100
EKVX	>100	>100	>100
HOP-62	73.1	>100	48
HOP-92	41.2	>100	97.6
NCI-H226	>100	>100	>100
NCI-H23	>100	>100	86
NCI-H322M	>100	>100	>100
NCI-H460	>100	>100	7.11
NCI-H522	>100	>100	>100
Colon cancer			
COLO 205	>100	>100	5.98
HCC-2998	>100	>100	6.99
HCT-116	45.6	>100	8.8
HCT-15	>100	>100	>100
HT29	>100	>100	6.91
KM12	>100	>100	8.05
SW620	>100	>100	—
Melanoma			
LOX IMVI	>100	>100	6.21
MALME-3M	>100	>100	>100
M14	78.6	>100	93.8
SK-MEL-2	>100	>100	>100
SK-MEL-28	48.7	>100	28.3
SK-MEL-5	>100	>100	13.6
UACC-257	>100	>100	>100
UACC-62	>100	>100	>100
CNS cancer			
SF-268	92.5	>100	>100
SF-295	>100	16.4	>100
SF-539	>100	>100	94.3
SNB-19	>100	>100	>100
SNB-75	>100	>100	>100
U251	76.0	>100	>100
Ovarian cancer			
IGROV1	79.2	>100	>100
OVCAR-3	91.3	>100	98.2
OVCAR-4	>100	>100	>100
OVCAR-5	>100	>100	>100
OVCAR-8	>100	>100	>100
SK-OV-3	>100	>100	>100
Renal cancer			
786-0	17.9	>100	6.75
A498	>100	>100	19.5
ACHN	>100	>100	>100
CAKI-1	>100	>100	>100
RXF393	19.5	>100	7.5
SN12C	>100	>100	6.29
TK-10	>100	>100	>100
UO-31	71.7	>100	>100
Breast cancer			
BT-549	>100	>100	>100
HS578T	>100	>100	—
MCF7	>100	>100	>100
MDA-MB-231/ATCC	>100	>100	28.3
MDA-MB-468	>100	>100	>100
T-47D	>100	>100	>100
Prostate cancer			
DU-145	12.5	>100	18.7
PC-3	78.4	>100	>100

[a] LC_{50} = concentration required to reduce total cell count by 50%. Calculated as $[(T_i - T_z)/T_z)] \times 100 = -50$, where T_z = absorbance at $t = 0$; T_i = absorbance at $t = 48$ h.



Scheme 1. Reagents and conditions: (a) $SOCl_2/CH_3CN$, rt, 15 h; (b) NaN_3/DMF, 150 °C, 1 h; (c) TBSCl/DMF, rt, 16 h; (d) PhN=C=O/CH_2Cl_2, rt, 5d; (e) H_2/Pd–C/EtOAc; (f) p-NO_2–$C_6H_4OCONHCH_3$/base/EtOAc, rt; (g) TFA/H_2O (9:1), rt, 3 h.

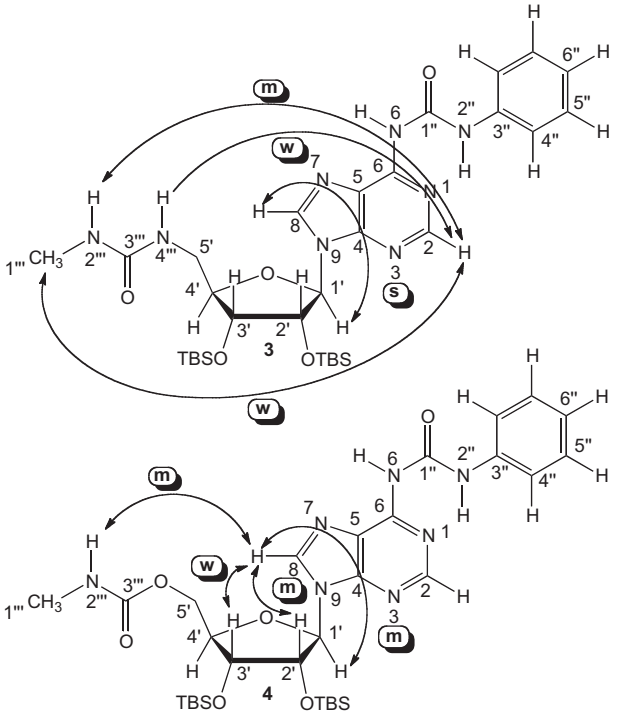


Figure 3. NOESY correlation data for compounds **3** and **4**: (s = strong, m = medium, w = weak).

Applying the observed $J_{1',2'}$ and $J_{3',4'}$ to Eq. 1 gives $K_{eq} = 11$ and 1.9 for compounds **3** and **4**, respectively.[10] These values correspond to the following N/S sugar conformational preferences for compounds **3** and **4**: **3** (8% N, 92% S), **4** (34% N, 66% S). The above

noted correlation between sugar conformations and the preferred glycosyl conformation (S correlates with *syn*; N correlates with *anti*),[7] indicates that compound **3** exists in a predominantly *syn* glycosyl conformation, while compound **4** is much more conformationally labile. The strong interactions between H8 and H1′ as well as between H2 and H2‴ in the NOESY spectrum for compound **3** also support the conclusion that the *syn* conformer is preferred. NOESY data for compound **4** show relatively weaker interactions between H8 and H1′ and stronger interactions between H8 and H2‴ and between H8 and H2′, all of which point to the *anti* conformation being more populated in compound **4** than in compound **3**. Taken together, these observations suggest that compound **3** enjoys a greater degree of conformational rigidity about the glycosidic linkage. The difference in conformational bias for compounds **3** and **4** could contribute to the difference in biological activities, as has been reported for conformationally biased nucleosides in a number of instances.[6]

In summary, we have discovered a broad spectrum anticancer nucleoside that exhibits low micromolar antiproliferative effects against a majority of the cell lines in the NCI-60. Selective toxicity toward colon cell lines was also exhibited. Compound **4**, a synthetically simpler, 5′-*N*-methylcarbamoyl analog of 5′-*N*-methylureido derivative **3**, was less active. The difference in activities may be due to the loss of one potential hydrogen bond donor in compound **4** relative to **3**, and/or may also be influenced by the greater conformational flexibility of compound **4**, as supported by NOESY and $^{3}J(^{1}H–^{1}H)$ coupling data. The selective toxicity of compound **3** may make it useful for future clinical applications. Appropriate derivatization of **3** could result in compounds with enhanced potency and selectivity. We are currently pursuing this line of investigation.

Acknowledgment

The NCI Developmental Therapeutics program is thanked for performance of antiproliferative and cytotoxicity evaluations.

Supplementary data

Supplementary data (detailed experimental procedures for compound **4**. NMR spectral data for compounds **3** and **4**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.003.

References and notes

1. Peterson, M. A.; Oliveira, M.; Christiansen, M. A.; Cutler, C. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6775.
2. Data from the NCI-60 screens is provided in the Supplementary data.
3. Average GI_{50} values are determined from NCI-60 testing results included in the Supplementary data. They do not take into account the cell lines with GI_{50} >100 μM.
4. Full experimental details for all new compounds can be found in the Supplementary data. ^{1}H and ^{13}C NMR and HRMS data for **4** are as follows: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.25 (br s, 1H), 9.86 (br s, 1H), 8.80 (s, 1H), 8.66 (s, 1H), 7.60 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.8 Hz, 2H), 7.16 (t, J = 7.3 Hz, 1H), 6.21 (d, J = 5.4 Hz, 1H), 5.82 (d, J = 4.0 Hz, 1H), 4.64 (t, J = 4.8 Hz, 1H), 4.50 (dd, J = 3.8, 12.8 Hz, 1H), 4.34 (t, J = 3.89 Hz, 1H), 4.31–4.29 (m, 2H), 2.47 (d, J = 5.0 Hz, 3H), 0.95 (s, 9H), 0.82 (s, 9H), 0.12 (s, 6H), 0.00 (s, 3H), −0.20 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.0, 153.4, 151.4, 150.8, 150.4, 143.9, 137.8, 129.3, 124.7, 121.6, 120.6, 87.8, 84.5, 77.4, 72.9, 63.6, 29.9, 27.1, 26.0, 25.9, 18.2, 18.0, −4.29, −4.61, −4.72, −5.15; MS (FAB) m/z 672.3356 (MH^+ [$C_{31}H_{50}N_7O_6Si_2$]) = 672.3353.
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10. Coupling constants for compounds **3** and **4** were as follows: compound **3** ($J_{1',2'}$ = 7.8 Hz, $J_{3',4'}$ = 0.7 Hz); **4** ($J_{1',2'}$ = 5.43 Hz, $J_{3',4'}$ = 2.83 Hz). See Supplementary data for complete list of coupling constants for compounds **3** and **4**.