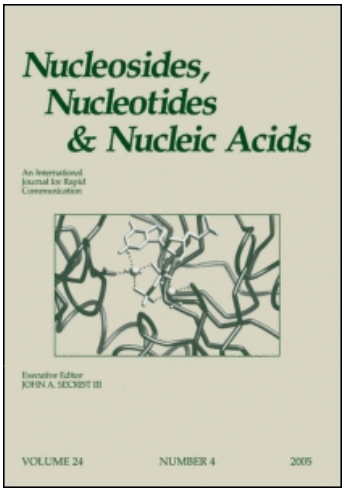

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Antiproliferative and Protein Kinase Binding Activities of Some N^6, 5'-bis-ureido 5'-Amino-5'-Deoxyadenosine Derivatives

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ANTIPROLIFERATIVE AND PROTEIN KINASE BINDING ACTIVITIES OF SOME N^6, 5′-BIS-UREIDO 5′-AMINO-5′-DEOXYADENOSINE DERIVATIVES

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□ *Two novel N^6,5′-bis-ureido 5′-amino-5′-deoxyadenosine derivatives are shown to inhibit tumor cell growth in the NCI 60 human tumor cell panel. Compounds **2c** and **2d** exhibited GI_{50} values of 1–6 μM in 35 and 14 cell lines, respectively. Compound **2c** was shown to selectively inhibit binding of protein kinases to immobilized ATP-binding site ligands via a competitive binding assay (11 of 353 protein kinases inhibited by ≥30% at 10 μM compound concentration). Enzyme inhibition assays revealed modest inhibition for PAK4 and FMS (21 and 17%, respectively). A brief SAR study suggests that a 2′-O-TBDMS is necessary for antiproliferative activity.*

Keywords protein kinase inhibitors; bis-ureidoadenosine derivatives; antiproliferative activity

INTRODUCTION

Interest in N^6-ureidoadenosine or 3′(5′)-ureido-3′(5′)-deoxyadenosine derivatives has been stimulated by the unique biological and physical properties exhibited by these compounds.[1–7] Various 3′-ureido derivatives have exhibited low- to submicromolar binding affinities for mutant human A_3(AR) receptors, an effect not observed with wild type A_3(AR), thus offering orthogonal and selective activation of the reengineered receptor via an organ-targeted "neoceptor" approach.[1] Select N^6-ureido-purine and -adenosine derivatives were prepared as early as the 1970's as potential antiproliferative agents,[2] and the N^6-carbamoyl moiety continues to be exploited as a versatile means of linking such useful functionalities to adenosine as fluorescent chromophores,[3] duplex stabilizing aryl groups,[4]

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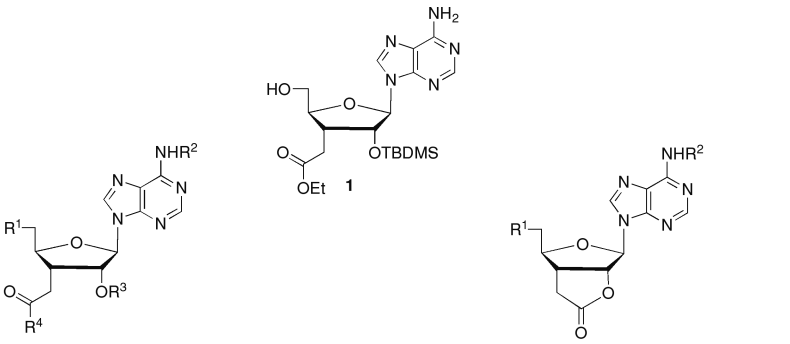


2a $R^1 = OH$; $R^2 = H$; $R^3 = H$; $R^4 = OH$
2b $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$; $R^3 = H$; $R^4 = ONa$
2c $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$; $R^3 = TBDMS$; $R^4 = OEt$
2d $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$; $R^3 = TBDMS$; $R^4 = NCH_3(OCH_3)$
2e $R^1 = N_3$; $R^2 = CONHC_6H_5$; $R^3 = H$; $R^4 = ONa$
2f $R^1 = N_3$; $R^2 = H$; $R^3 = H$; $R^4 = OH$
2g $R^1 = N_3$; $R^2 = CONHC_6H_5$
2h $R^1 = CH_3NHCONH$; $R^2 = CONHC_6H_5$

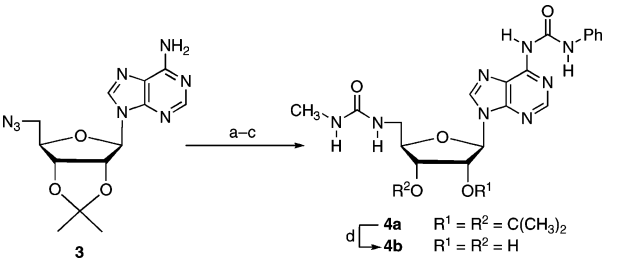
FIGURE 1 Putative HIV integrase inhibitors.

and reporter groups for spin-labeling studies.[5] 5′-Nitrosoureidoadenosine and other 5′-nitrosoureidonucleoside derivatives were prepared as potential antileukemia agents in the late 1970s. Negligible activities were reported for these compounds against murine L1210, and a 5′-*N*-methylureidoadenosine derivative was also ineffective against this cell line at concentrations as high as 60 μM.[6]

Recently, we reported the synthesis and biological activities of some 3′-carboxymethyl-3′-deoxyadenosine derivatives designed to bind to the active site of HIV integrase (compounds **2a–h**, Figure 1).[7] A key intermediate in the synthesis of compounds **2a–h** was compound **1** which was prepared via stereospecific reduction of 2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′-[(ethoxycarbonyl)methylene]adenosine following the procedure of Robins et al.[8–9] Evaluation of compounds **2b–h** in HIV replication or HIV integrase assays revealed that **2b–h** were ineffective as inhibitors of HIV replication, and 3′-endprocessing and strand transfer activities of HIV integrase were not inhibited at the concentrations tested. Compounds **2c**, **2d**, **2g**, and **2h** did however show promising activities ($IC_{50} = 21.9–58.5$ μM) against MT2 cells in vitro. These activities prompted an evaluation of compounds **2c**, **2d**, **2g**, and **2h** in the NCI 60 human tumor cell line panel, and the results of such screening along with protein kinase binding activities are presented in this article.

RESULTS AND DISCUSSION

Compounds **2c**, **2d**, **2g**, and **2h** were submitted to the National Cancer Institute Developmental Therapeutics Program for screening against the NCI 60 panel of human tumor cells in vitro.[10] Results of the single dose



SCHEME 1 Reagents and conditions: (a) PhN = C = O, CH_2Cl_2, 5 d; (b) H_2/Pd–C, EtOAc, 12 hours; (c) *p*-$NO_2C_6H_4OCONHCH_3$, Na_2CO_3, EtOAc, 4 hours; (d) TFA, CH_2Cl_2, H_2O, 4 hours.

(10 μM) initial screen are illustrated in Table 1. Compounds **2c** and **2d** were superior to lactones **2g** and **2h** in the single dose growth inhibition assay. Only one cell line (renal cancer RXF393) was inhibited to any appreciable extent by **2g** and **2h**. In contrast, numerous cell lines were inhibited by **2c** and **2d**. To determine whether the inferior antiproliferative activities exhibited by compounds **2g** and **2h** reflected a structural requirement for the 2′-*O*-TBDMS, compounds **4a** and **4b** were prepared and tested (Scheme 1). The antiproliferative activities for **2g**(**h**) and **4a**(**b**) suggests that the 2′-*O*-TBDMS is a minimal requirement for anticancer activity (Table 2).

On the basis of the results from the single dose assay, compounds **2c** and **2d** were selected for multi-dose testing (Tables 3 and 4). In the multi-dose assays, compounds **2c** and **2d** exhibited low μM inhibition of all six leukemias tested. Numerous additional cell lines from the other subclasses were also inhibited at the low μM level. Compound **2d** appeared somewhat more toxic than **2c** toward the leukemia cell lines, and LC_{50} values for **2d** ranged from 9.43–59.0 μM for leukemias SR, HL-60(TB), and RPMI-8226. In contrast, LC_{50} values for compound **2c** were >100 μM for all leukemias except RPMI-8226. LC_{50} values for RPMI-8226 were identical for both **2c** and **2d** (59.0 μM). Compound **2c** was more cytotoxic than **2d** against all cell lines tested (16 cell lines showed LC_{50} values <100 μM for **2c** compared to only three cell lines for compound **2d**), but a majority of cell lines for **2c** showed LC_{50} values >100.0 μM. Compound **2c** also exhibited greater efficacy in cell growth inhibition than **2d**, with 35 cell lines having GI_{50} values $\leq$6 μM for **2c** compared to only 14 cell lines inhibited at similar levels for compound **2d**.

A COMPARE[11] analysis of the GI_{50} data for compound **2c** suggested that protein kinases might be molecular targets for this compound. Accordingly, compound **2c** was screened against a commercially available panel of 353 protein kinases (KinomeScan, Ambit Biosciences, San Diego, CA, USA),[12] and the binding affinities of compound **2c** for these kinases were determined. The KinomeScan assay is a competitive binding assay involving phage-display of protein kinases and immobilized ATP-binding

TABLE 1 Results of single dose growth inhibition assay (GI Percent at 10 μM)[a]

Cell Line	2c	2d	2g	2h	Cell Line	2c	2d	2g	2h
Leukemia					**CNS Cancer**				
CCRF-CEM	50	—	92	94	SF-268	59	33	103	100
HL-60(TB)	45	−33	87	83	SF-295	16	−41	100	103
K-562	59	16	93	81	SF-539	53	0	98	104
MOLT-4	50	−11	85	70	SNB-19	94	37	106	103
RPMI−8226	11	−75	98	105	SNB-75	63	−7	94	97
SR	28	−56	90	90	U251	34	−15	110	96
Non-Small Cell Lung Cancer					**Ovarian Cancer**				
A549/ATCC	22	2	95	120	IGROV1	51	−37	91	92
EKVX	64	21	103	102	OVCAR-3	80	8	95	92
HOP-62	79	60	98	94	OVCAR-4	61	19	106	106
HOP-92	63	−34	87	71	OVCAR-5	100	44	96	97
NCI-H226	76	41	106	99	OVCAR-8	65	20	103	96
NCI-H23	92	48	105	90	SK-OV-3	94	41	106	102
NCI-H322M	91	79	100	101	**Renal Cancer**				
NCI-H460	48	18	112	114	786−0	76	48	105	98
NCI-H522	80	66	107	106	A498	71	46	100	94
Colon Cancer					ACHN	69	12	101	95
COLO 205	43	−100	104	110	CAKI-1	82	43	101	100
HCC-2998	62	−13	106	93	RXF393	−45	−68	−29	−23
HCT-116	17	17	100	98	SN12C	61	17	118	113
HCT-15	56	12	91	92	TK-10	44	7	101	99
HT29	27	−26	109	110	UO-31	57	−7	79	64
KM12	43	17	106	106	**Breast Cancer**				
SW620	71	24	105	106	BT-549	82	26	104	100
Melanoma					HS578T	44	4	111	105
LOX IMVI	50	19	93	63	MCF7	18	3	92	98
MALME-3M	55	12	97	100	MDA-MB-231/ATCC	51	15	123	99
M14	67	34	107	105	MDA-MB-435	54	8	109	102
SK-MEL-2	54	−4	91	70	NCI/ADR-RES	86	54	102	97
SK-MEL-28	84	8	118	107	T-47D	27	−34	103	92
SK-MEL-5	58	26	106	110	**Prostate Cancer**				
UACC-257	88	6	122	125	DU-145	63	25	109	110
UACC-62	80	34	107	99	PC-3	47	−21	97	100

[a]Growth inhibition percent calculated as:
$[(T_i\text{-}T_Z)/C\text{-}T_Z)] \times 100$ for $T_i \geq T_z$
$[(T_i\text{-}T_z)/T_z)] \times 100$ 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.

site ligands.[13] Binding of phage-displayed protein kinases to immobilized ATP-binding site ligands was inhibited by compound **2c** by ≥30% for 11 of the 353 protein kinases evaluated. A majority of the kinases were unaffected by **2c** and binding inhibition of ≤10% was observed for >225 kinases (Figure 2A). Such selectivity is a desirable property suggestive of the potential utility of derivatives of compound **2c** in therapeutic applications.[14] Kinases for which binding was inhibited by ≥30% included EGFR, TYK2, FLT3, CSNK2A2, PAK3, MARK3, BTK, IKK-α, CSNK1G2, RPS6KA1, and BMPR1B,

TABLE 2 Results of single dose growth inhibition assay (GI Percent at 10 μM)[a]

Cell Line	4a	4b	Cell Line	4a	4h
Leukemia			**CNS Cancer**		
CCRF-CEM	95	100	SF-268	91	99
HL-60(TB)	84	79	SF-295	119	123
K-562	90	60	SF-539	84	91
MOLT-4	103	—	SNB-19	85	89
RPMI-8226	87	90	SNB-75	60	66
Non-Small Cell Lung Cancer			U251	94	86
A549/ATCC	103	99	**Ovarian Cancer**		
EKVX	102	113	OVCAR-3	89	85
HOP-62	99	96	OVCAR-4	90	92
HOP-92	14	71	OVCAR-5	109	102
NCI-H226	109	99	OVCAR-8	100	105
NCI-H23	87	95	SK-OV-3	79	77
NCI-H322M	101	95	**Renal Cancer**		
NCI-H460	101	101	786-0	105	106
NCI-H522	104	92	A498	87	103
Colon Cancer			ACHN	105	99
HCC-2998	96	78	CAKI-1	73	55
HCT-116	81	89	RXF393	97	114
HCT-15	98	—	SN12C	95	96
HT29	99	97	TK-10	134	152
KM12	90	89	UO-31	74	98
SW620	91	98	**Breast Cancer**		
Melanoma			BT-549	72	—
LOX IMVI	100	99	HS578T	102	93
MALME-3M	76	97	MCF7	85	91
M14	89	108	MDA-MB-231/ATCC	107	90
SK-MEL-2	104	117	MDA-MB-468	100	98
SK-MEL-28	92	100	T-47D	75	78
SK-MEL-5	86	94	**Prostate Cancer**		
UACC-257	107	105	DU-145	94	95
UACC-62	92	88	PC-3	71	85

[a]Growth inhibition percent calculated as:
$[(T_i\text{-}T_z)/C\text{-}T_z)] \times 100$ for $T_i \geq T_z$
$[(T_i\text{-}T_z)/T_z)] \times 100$ 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.

each of which has been implicated in various forms of cancer (Figure 2B). Binding inhibition was greatest for BMPR1B (or ALK6), a recently implicated modulator of estrogen receptor positive breast cancer.[15,16] The selective inhibition of binding of ALK6 compared to other members of the ALK family of protein kinases suggests that **2c** might be a useful probe for elucidating the role played by ALK6 in BMP-mediated signaling (Figure 2C).[17] Selective inhibition of binding was also observed for other protein kinase families (e.g., p38 and PAK kinase families; Figure 3).

Compound **2c** was also screened for its ability to inhibit a panel of cancer-related protein kinases (Figure 4). Activities for several of the protein

TABLE 3 Results of multi dose growth inhibition assay for compound **2c** (μM)[a]

Cell Line	GI_{50}	TGI	LC_{50}	Cell Line	GI_{50}	TGI	LC_{50}
Leukemia				**CNS Cancer**			
CCRF-CEM	6.69	88.6	> 100.0	SF-268	6.53	27.0	92.5
HL-60(TB)	3.01	—	> 100.0	SF-295	5.73	> 100.0	> 100.0
K-562	3.59	32.9	> 100.0	SF-539	5.19	> 100.0	> 100.0
MOLT-4	2.39	23.3	> 100.0	SNB-19	29.0	> 100.0	> 100.0
RPMI-8226	1.09	4.57	59.0	SNB-75	4.56	> 100.0	> 100.0
SR	2.23	7.07	> 100.0	U251	4.69	20.9	76.0
Non-Small Cell Lung Cancer				**Ovarian Cancer**			
A549/ATCC	4.18	19.2	79.1	IGROV1	3.85	18.0	79.2
EKVX	17.7	> 100.0	> 100.0	OVCAR-3	4.59	17.2	91.3
HOP-62	8.96	26.4	73.1	OVCAR-4	1.23	> 100.0	> 100.0
HOP-92	< 0.01	—	41.2	OVCAR-5	31.1	> 100.0	> 100.0
NCI-H226	> 109.0	> 100.0	> 100.0	OVCAR-8	4.92	77.2	> 100.0
NCI-H23	33.3	> 100.0	> 100.0	SK-OV-3	21.0	> 100.0	> 100.0
NCI-H322M	> 100.0	> 100.0	> 100.0	**Renal Cancer**			
NCI-H460	5.54	2.5	> 100.0	786-0	2.00	5.21	17.9
NCI-H522	4.36	85.7	> 100.0	A498	3.34	17.1	> 100.0
Colon Cancer				ACHN	8.55	> 100.0	> 100.0
COLO 205	3.84	> 100.0	> 100.0	CAKI-1	29.7	> 100.0	> 100.0
HCC-2998	> 100.0	> 100.0	> 100.0	RXF393	2.01	4.63	19.5
HCT-116	3.20	16.1	45.6	SN12C	9.10	> 100.0	> 100.0
HCT-15	8.50	> 100.0	> 100.0	TK-10	12.4	40.5	> 100.0
HT29	4.20	> 100.0	> 100.0	UO-31	12.1	29.5	71.7
KM12	3.95	20.9	> 100.0	**Breast Cancer**			
SW620	4.80	28.4	> 100.0	MCF7	3.42	45.1	> 100.0
Melanoma				NCI/ADR-RES	> 100.0	> 100.0	> 100.0
LOX IMVI	5.46	> 100.0	> 100.0	MDA-MB-231/ATCC	3.96	41.3	> 100.0
MALME-3M	10.3	> 100.0	> 100.0	HS578T	3.60	53.6	> 100.0
M14	2.51	11.6	7.86	MDA-MB-435	6.21	> 100.0	> 100.0
SK-MEL-2	5.42	33.1	> 100.0	BT-549	> 100.0	> 100.0	> 100.0
SK-MEL-28	6.85	20.2	48.7	T-47D	2.55	> 100.0	> 100.0
SK-MEL-5	4.34	> 100.0	> 100.0	**Prostate Cancer**			
UACC-257	5.68	> 100.0	> 100.0	PC-3	2.25	4.85	12.5
UACC-62	> 100.0	> 100.0	> 100.0	DU-145	4.97	19.4	78.4

[a]GI_{50} = concentration at which cell growth is inhibited by 50%; TGI = concentration required to achieve total growth inhibition; LC_{50} = concentration required to achieve 50% reduction in measured protein after 48 h test period. TGI signifies a cytostatic effec; LC_{50} signifies a cytotoxic effect.

$[(T_i\text{-}T_z)/C\text{-}T_z)] \times 100 = 50$ for GI_{50}

$T_i = T_z$ for TGI

$[(T_i\text{-}T_z)/T_z)] \times 100 = -50$ for LC_{50}

Where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h; C = absorbance of control at t = 48 h.

kinases were modestly enhanced at 20 μM compound concentration and two of the kinases (FMS and PAK4) were inhibited.

CONCLUSIONS

Novel N^6,5′-bis-ureido 5′-amino-5′-deoxyadenosine derivatives **2c** and **2d** inhibited proliferation of several human tumor cell lines in vitro. GI_{50} values

TABLE 4 Results of multi dose growth inhibition assay for compound **2d** (μM)[a]

Cell Line	GI_{50}	TGI	LC_{50}	Cell Line	GI_{50}	TGI	LC_{50}
Leukemia				**CNS Cancer**			
CCRF-CEM	6.37	> 100.0	> 100.0	SF-268	8.29	> 100.0	> 100.0
HL-60(TB)	1.81	4.34	16.4	SF-295	9.09	> 100.0	> 100.0
K-562	3.12	> 100.0	> 100.0	SF-539	22.3	> 100.0	> 100.0
MOLT-4	2.23	2.23	> 100.0	SNB-19	> 100.0	> 100.0	> 100.0
RPMI-8226	1.58	1.58	59.0	SNB-75	12.7	> 100.0	> 100.0
SR	1.27	1.27	9.43	U251	5.66	> 100.0	> 100.0
Non-Small Cell Lung Cancer				**Ovarian Cancer**			
A549/ATCC	9.35	> 100.0	> 100.0	IGROV1	3.72	65.7	> 100.0
EKVX	26.4	> 100.0	> 100.0	OVCAR-3	7.11	> 100.0	> 100.0
HOP-62	24.9	> 100.0	> 100.0	OVCAR-4	53.0	> 100.0	> 100.0
HOP-92	2.71	24.3	> 100.0	OVCAR-5	38.2	> 100.0	> 100.0
NCI-H226	41.9	> 100.0	> 100.0	OVCAR-8	9.02	> 100.0	> 100.0
NCI-H23	> 100.0	> 100.0	> 100.0	SK-OV-3	52.7	> 100.0	> 100.0
NCI-H322M	> 100.0	> 100.0	> 100.0	**Renal Cancer**			
NCI-H460	7.49	> 100.0	> 100.0	786-0	9.01	> 100.0	> 100.0
NCI-H522	11.1	> 100.0	> 100.0	A498	3.87	40.3	> 100.0
Colon Cancer				ACHN	14.4	> 100.0	> 100.0
COLO 205	12.3	> 100.0	> 100.0	CAKI-1	53.8	> 100.0	> 100.0
HCC-2998	30.6	> 100.0	> 100.0	RXF393	9.74	38.0	> 100.0
HCT-116	4.20	> 100.0	> 100.0	SN12C	85.3	> 100.0	> 100.0
HCT-15	6.47	> 100.0	> 100.0	TK-10	20.5	> 100.0	> 100.0
HT29	5.37	> 100.0	> 100.0	UO-31	7.79	> 100.0	> 100.0
KM12	23.9	> 100.0	> 100.0	**Breast Cancer**			
SW620	> 100.0	> 100.0	> 100.0	MCF7	5.59	> 100.0	> 100.0
Melanoma				NCI/ADR-RES	> 100.0	> 100.0	> 100.0
LOX IMVI	7.30	> 100.0	> 100.0	MDA-MB-231/ATCC	12.3	> 100.0	> 100.0
MALME-3M	14.1	> 100.0	> 100.0	HS578T	5.79	> 100.0	> 100.0
M14	15.2	> 100.0	> 100.0	MDA-MB-435	10.9	> 100.0	> 100.0
SK-MEL-2	14.9	83.1	> 100.0	BT-549	29.0	> 100.0	> 100.0
SK-MEL-28	7.77	> 100.0	> 100.0	T-47D	13.9	> 100.0	> 100.0
SK-MEL-5	5.81	> 100.0	> 100.0	**Prostate Cancer**			
UACC-257	22.6	> 100.0	> 100.0	PC-3	—	—	—
UACC-62	41.9	> 100.0	> 100.0	DU-145	1.66	> 100.0	> 100.0

[a]GI_{50} = concentration at which cell growth is inhibited by 50%; TGI = concentration required to achieve total growth inhibition; LC_{50} = concentration required to achieve 50% reduction in measured protein after 48 h test period. TGI signifies a cytostatic effec; LC_{50} signifies a cytotoxic effect.

$[(T_i\text{-}T_z)/C\text{-}T_z)] \times 100 = 50$ for GI_{50}

$T_i = T_z$ for TGI

$[(T_i\text{-}T_z)/T_z)] \times 100 = -50$ for LC_{50}

Where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h; C = absorbance of control at t = 48 h.

in the low (1–6) μM region were exhibited in over half of the NCI 60 panel for compound **2c**. A competitive binding assay suggests that **2c** may bind to the ATP-binding site of several protein kinases. ALK6, a recently implicated modulator of estrogen positive breast cancer, was inhibited most significantly in this assay. Inhibition of phosphorylation of FMS and PAK4, two cancer-related protein kinases, was modest (17% and 21%, respectively)

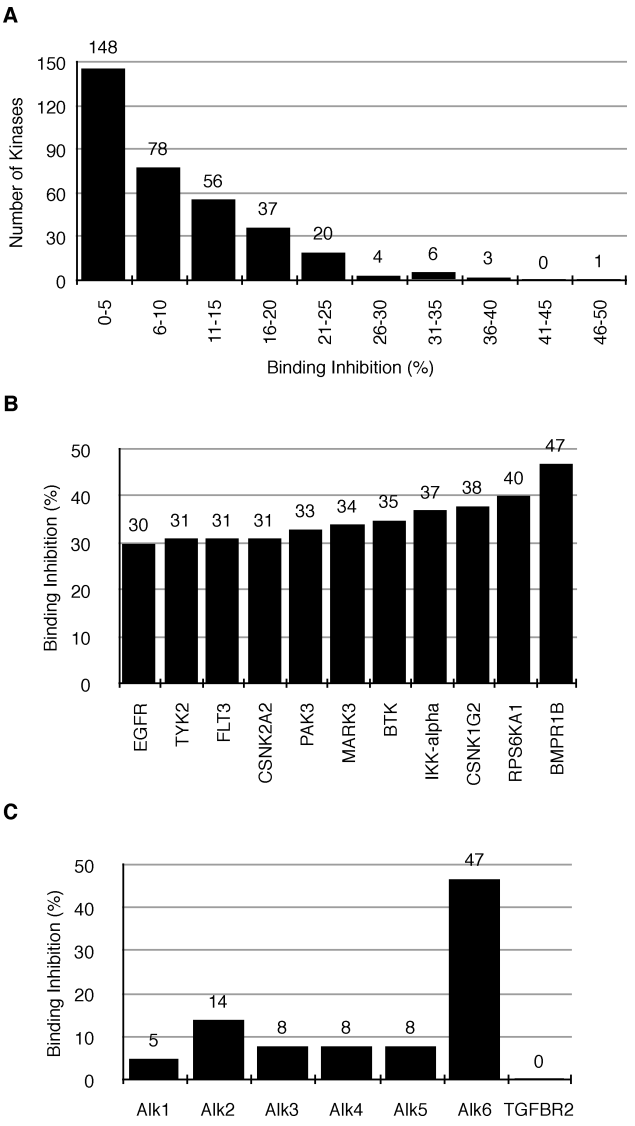


FIGURE 2 Inhibition of binding of protein kinases to immobilized ATP-binding site ligands by compound **2c** (10 μM). (A) Results of 353 member KinomeScan competitive binding assay; (B) Protein kinases inhibited by ≥30%; (C) Selective inhibition of binding of ALK6.

suggesting that the primary mechanism of antiproliferative activity may not be attributable to inhibition of these two enzymes. A minimal requirement for antiproliferative activity appears to be the presence of the sterically bulky 2′-*O*-TBDMS group. Future SAR studies will focus on the impact of the N^6 and 5′-ureido groups.

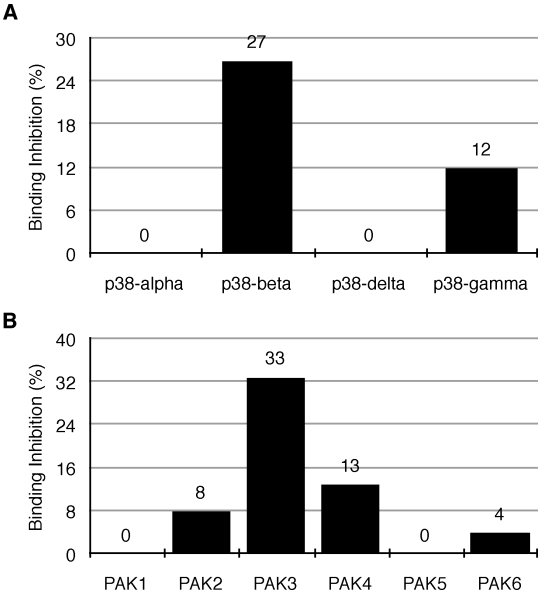


FIGURE 3 Inhibition of binding of protein kinases to immobilized ATP-binding site ligands by compound **2c** (10 μM). (A) Selective inhibition of p38 kinase family; (B) Selective inhibition of PAK kinase family.

Compound Preparation

5′-Deoxy-2′,3′-O-isopropylidene-5′-[(N-methylcarbamoyl)amino]-N^6-(N-phenylcarbamoyl)adenosine (4a)

To a flame dried flask containing compound **3** (454 mg, 1.37 mmol) was added phenylisocyanate (190 mg, 1.6 mmol) in CH_2Cl_2 (16 mL). The resulting solution was stirred protected from moisture until TLC indicated that starting material had been consumed (5 days). The crude solution was added directly to a flash chromatography column and eluted (50 → 75%EtOAc/hexanes → 10%MeOH/EtOAc). Appropriate fractions were pooled and volatiles were evaporated under reduced pressure to give 5′-azido-5′-deoxy-2′,3′-*O*-isopropylidene-N^6-(*N*-phenylcarbamoyl) adenosine (491 mg, 79%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.71 (s, 1H), 8.66 (s, 1H), 8.25 (s, 1H), 8.21 (s, 1H), 7.66 (d, J = 8.5 Hz, 2H), 7.39 (t, J = 8.0 Hz, 2H), 7.15 (t, J = 7.3 Hz, 1H), 6.19 (d, J = 2.5 Hz, 1H), 5.44 (dd, J = 6.3, 2.3 Hz, 1H), 5.07 (dd, J = 6.0, 3.5 Hz, 1H), 4.43 (dd, J = 9.0, 5.0 Hz, 1H), 3.63 (dd, J = 9.5, 4.8 Hz, 2H), 1.65 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.2, 151.1, 150.2, 149.9, 142.1, 137.9, 129.1, 124.1, 121.1, 120.4, 115.1, 90.7, 85.3, 84.1, 81.8, 52.3, 27.2, 25.4; MS (FAB) *m/z* 452.17923 (MH^+ [$C_{20}H_{22}N_9O_4$] = 452.17948).

A solution of 5′-azido-5′-deoxy-2′,3′-*O*-isopropylidene-N^6-(*N*-phenylcarbamoyl)adenosine (70 mg, 0.16 mmol) and 10% Pd–C (40 mg) in EtOAc (10 mL) was vigorously stirred for 15 hours under an atmosphere of H_2

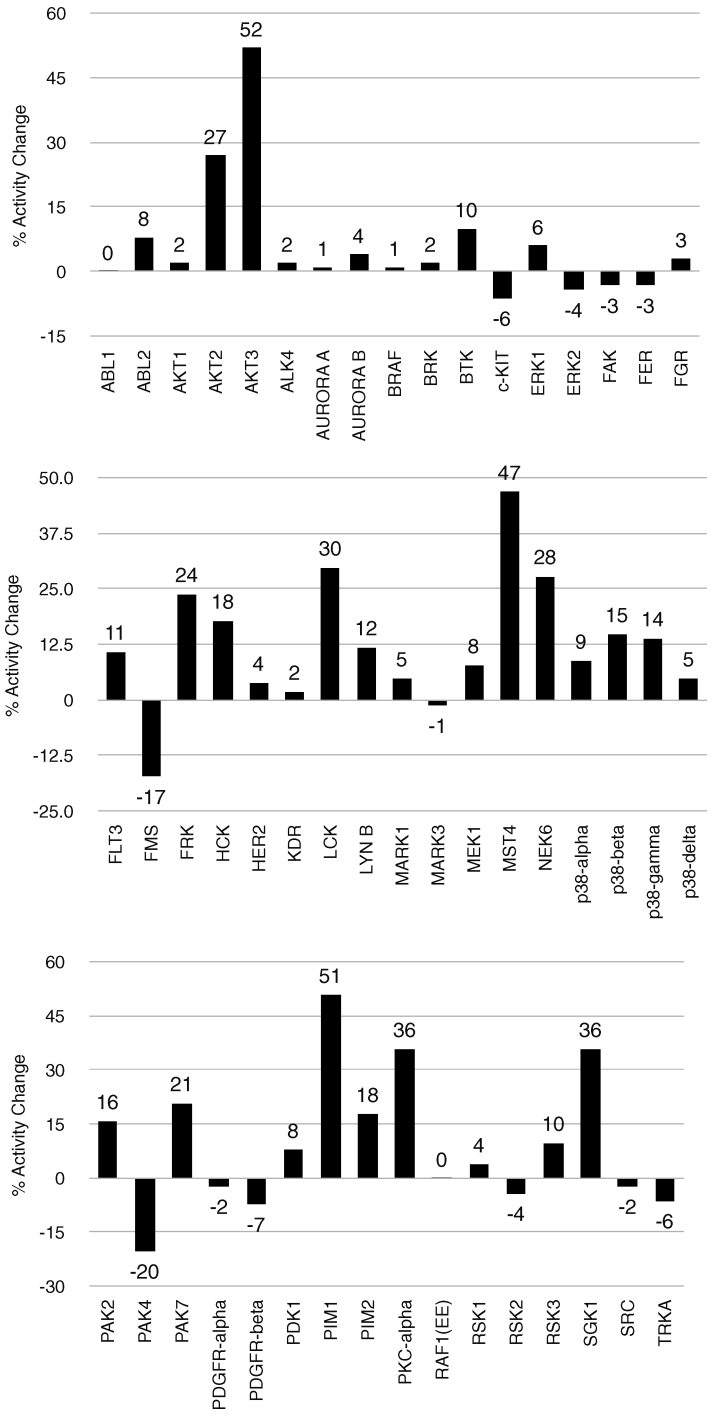


FIGURE 4 Percent activity change in cancer-related protein kinases treated with **2c** (20 μM).

(balloon pressures). *p*-Nitrophenyl *N*-methylcarbamate (43 mg, 0.22 mmol) and anhydrous Na_2CO_3 (45 mg, 0.43 mmol) were added, and the resulting mixture was stirred for 4 hours under N_2. Solids were removed via filtration (celite/EtOAc), and volatiles were evaporated under reduced pressure. The crude residue was chromatographed (5 → 10% MeOH/CH_2Cl_2) to give **4a** (65 mg, 87%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.10 (s, 1H), 9.79 (s, 1H), 8.69 (s, 1H), 8.68 (s, 1H), 7.56 (dd, J = 8.8, 0.8 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 7.20 (t, J = 8.0 Hz, 1H), 6.15 (d, J = 4.0 Hz, 1H), 5.84 (m, 1H), 5.27 (dd, J = 6.3, 3.8 Hz, 1H), 4.98 (dd, J = 6.3, 2.3 Hz, 1H), 4.77 (m, 1H), 4.52 (dd, J = 6.8, 2.8 Hz, 1H), 3.74 (ddd, J = 13.8, 7.4, 4.1 Hz, 1H), 3.38 (dt, J = 3.8, 14.8 Hz, 1H), 2.56 (d, J = 4.5 Hz, 3H), 1.65 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 152.6, 150.9, 150.34, 150.30, 143.3, 137.2, 129.2, 124.9, 121.5, 121.2, 114.6, 91.6, 85.9, 83.9, 81.6, 41.8, 27.4, 26.9, 25.4; MS (FAB) *m/z* 483.2099 (MH^+ [$C_{22}H_{27}N_8O_5$] = 483.2099).

5′-Deoxy-5′-[(N-methylcarbamoyl)amino]-N^6-(N-phenylcarbamoyl)adenosine (4b)

A solution of **4a** (10 mg, 0.021 mmol), TFA (100 μL), and H_2O (25 μL) in CH_2Cl_2 (500 μL) was vigorously stirred at ambient temperature until TLC indicated complete conversion to baseline product (4 hours). Volatiles were removed under reduced pressure ($\leq$25°C) and the crude was purified via flash chromatography (EtOAc/iPrOH/H_2O, 4:1:2) to give **4b** (7 mg, 75%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.75 (s, 1H), 10.21 (s, 1H), 8.71 (s, 1H), 8.70 (s, 1H), 7.61 (d, J = 7.5 Hz, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.08 (t, J = 7.5 Hz, 1H), 6.10 (s, 1H), 5.97 (d, J = 6.0 Hz, 1H), 5.80 (s, 1H), 4.67 (t, J = 5.5 Hz, 1H), 4.09 (dd, J = 3.5, 5.0 Hz, 1H), 3.94–3.91 (m, 1H), 3.41 (dd, J = 14.3, 4.3 Hz, 1H), 3.26 (dd, J = 14.3, 6.3 Hz, 1H), 2.49 (s, 3H; overlaps with DMSO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1, 151.4, 151.2, 151.1, 150.2, 143.4, 138.9, 129.4, 123.7, 121.1, 119.9, 87.9, 84.9, 73.6, 71.6, 42.2, 26.8; MS (FAB) *m/z* 443.17757 (MH^+ [$C_{19}H_{23}N_8O_5$] = 443.17859).

KinomeScan Competitive Binding Assay

This assay is based on ligand-affinity/protein kinase phage display and was employed essentially as described by Lockhart and co-workers.[13] In this assay, protein kinases are cloned into T7 bacteriophage which express the kinase fusion proteins on the phage capsid. T7 kinase-tagged phage are then screened for binding to ATP-binding-site ligands that have been immobilized on a solid support. Phage are screened for binding to the anchored ligands both in the presence of test compound and in its absence (control). Elution of the bound phage by free ligand (ATP-binding site ligand that is not immobilized on a solid support) followed by determination of the phage titre provides a reliable measure of the ability of test compounds to block binding of target kinases to resin-bound ATP-binding-site ligands.

This method has allowed rapid mapping of small molecule interactions with ATP-binding sites across a broad cross-section of disease related protein kinases and has been validated as a reliable tool for identifying ligands with strong affinities for ATP-binding sites in numerous protein kinases.[13] Analyses were performed in duplicate and the values reported in Figure 2A–C are averages for the two experiments.

Kinases screened in this assay included: AAK1, ABL1, ABL1(E255K), ABL1(F317I), ABL1(F317L), ABL1(H396P), ABL1(M351T), ABL1 (Q252H), ABL1(T315I), ABL1(Y253F), ABL2, ACVR1, ACVR1B, ACVR2A, ACVR2B, ACVRL1, ADCK3, ADCK4, AKT1, AKT2, AKT3, ALK, AMPK-alpha1, AMPK-alpha2, ANKK1, ARK5, AURKA, AURKB, AURKC, AXL, BIKE, BLK, BMPR1A, BMPR1B, BMPR2, BMX, BRAF, BRAF(V600E), BRSK1, BRSK2, BTK, CAMK1, CAMK1D, CAMK1G, CAMK2A, CAMK2B, CAMK2D, CAMK2G,

CAMK4, CAMKK1, CAMKK2, CDC2L1, CDC2L2, CDK11, CDK2, CDK3, CDK5, CDK7, CDK8, CDK9, CDKL2, CHEK1, CHEK2, CIT, CLK1, CLK2, CLK3, CLK4, CSF1R, CSK, CSNK1A1L, CSNK1D, CSNK1E, CSNK1G1, CSNK1G2, CSNK1G3, CSNK2A1, CSNK2A2, DAPK1, DAPK2, DAPK3, DCAMKL1, DCAMKL2, DCAMKL3, DDR1, DDR2, DLK, DMPK, DMPK2, DRAK1, DRAK2, DYRK1B, EGFR, EGFR(E746-A750del), EGFR(G719C), EGFR(G719S), EGFR(L747-E749del, A750P), EGFR(L747-S752del, P753S), EGFR(L747-T751del,Sins), EGFR(L858R), EGFR(L861Q), EGFR(S752-I759del), EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHB1, EPHB2, EPHB3, EPHB4, ERBB2, ERBB4, ERK1, ERK2, ERK3, ERK4, ERK5, ERK8, FER, FES, FGFR1, FGFR2, FGFR3, FGFR3(G697C), FGFR4, FGR, FLT1, FLT3, FLT3(D835H), FLT3(D835Y), FLT3(ITD), FLT3(K663Q), FLT3(N841I), FLT4, FRK, FYN, GAK, GCN2(Kin.Dom.2,S808G), GSK3A,

GSK3B, HCK, HIPK1, IGF1R, IKK-alpha, IKK-beta, IKK-epsilon, INSR, INSRR, IRAK3, ITK, JAK1 (Kin.Dom.1), JAK1(Kin.Dom.2), JAK2(Kin.Dom.2), JAK3(Kin.Dom.2), JNK1, JNK2, JNK3, KIT, KIT (D816V), KIT(V559D), KIT(V559D,T670I), KIT(V559D,V654A), LATS1, LATS2, LCK, LIMK1, LIMK2, LKB1, LOK, LTK, LYN, MAP3K3, MAP3K4, MAP3K5, MAP4K1, MAP4K2, MAP4K3, MAP4K4, MAP4K5, MAPKAPK2, MAPKAPK5, MARK1, MARK2, MARK3, MARK4, MEK1, MEK2, MEK3, MEK4, MEK6, MELK, MERTK, MET, MINK, MKNK1, MKNK2, MLCK, MLK1, MLK2, MLK3, MRCKA,

MRCKB, MST1, MST1R, MST2, MST3, MST4, MUSK, MYLK, MYLK2, MYO3A, MYO3B, NDR2, NEK1, NEK2, NEK5, NEK6, NEK7, NEK9, NLK, p38-alpha, p38-beta, p38-delta, p38-gamma, PAK1, PAK2, PAK3, PAK4, PAK6, PAK7/PAK5, PCTK1, PCTK2, PCTK3, PDGFRA, PDGFRB, PDPK1, PFTAIRE2, PFTK1, PHKG1, PHKG2, PIK3C2B, PIK3CA, PIK3CA(E545K), PIK3CB, PIK3CD, PIK3CG, PIM1, PIM2, PIM3, PIP5K1A, PIP5K2B, PKAC-alpha, PKAC-beta, PKMYT1, PKN1, PKN2, PLK1, PLK3, PLK4,

PRKCD, PRKCE, PRKCH, PRKCQ, PRKD1, PRKD2, PRKD3, PRKG1, PRKG2, PRKR, PRKX, PTK2, PTK2B, PTK6, RAF1, RET, RET(M918T), RET(V804L), RET(V804M), RIOK1, RIOK2, RIOK3, RIPK1, RIPK2, RIPK4, ROCK2, ROS1, RPS6KA1(Kin.Dom.1), RPS6KA1 (Kin.Dom.2), RPS6KA2(Kin.Dom.1), RPS6KA2(Kin.Dom.2), RPS6KA3(Kin.Dom.1), RPS6KA4

(Kin.Dom.1), RPS6KA4(Kin.Dom.2), RPS6KA5(Kin.Dom.1), RPS6KA5 (Kin.Dom.2), RPS6KA6 (Kin.Dom.1), RPS6KA6(Kin.Dom.2), SgK085, SgK110, SLK, SNARK, SNF1LK, SNF1LK2, SRC, SRMS, SRPK1, SRPK2, SRPK3, STK16, STK33, STK35, STK36, SYK, TAK1, TAOK1, TAOK3, TEC, TESK1, TGFBR1, TGFBR2, TIE1, TIE2, TLK1, TLK2, TNIK, TNK1, TNK2, TNNI3K, TRKA, TRKB, TRKC, TSSK1, TTK, TXK, TYK2(Kin.Dom.1), TYK2(Kin.Dom.2), TYRO3, ULK1, ULK2, ULK3, VEGFR2, WEE1, WEE2, YANK2, YANK3, YES, YSK1, ZAK, ZAP70.

Kinase Inhibition Assay

The various protein kinase targets employed in the kinase inhibition assay were cloned, expressed, and purified in-house at SignalChem Inc. (Richmond, BC, Canada) using proprietary methods. Quality control testing is routinely performed on each of the SignalChem targets to ensure compliance to acceptable standards. Protein substrates employed in the target profiling process were synthesized internally. ^{33}P-ATP was purchased from PerkinElmer. All other materials were of standard grade. Compound **2c** was supplied to SignalChem in a powder form. It was reconstituted in DMSO to form a stock solution which was then diluted with 10% DMSO to form a working stock solution (100 μM) that was then profiled against the various protein kinase targets. Protein kinase assays were performed in triplicate at ambient temperature for 20–40 minutes (depending on the target) in a final volume of 25 μl according to the following assay reaction recipe: Component 1: 5 μl of diluted active protein kinase target (~10–40 nM final protein concentration in the assay); Component 2: 5 μl of stock solution of substrate (1–5 μg of peptide or protein substrate); Component 3: 5 μl of kinase assay buffer or protein kinase activator in kinase assay buffer; Component 4: 5 μl of Compound **2c** (100 μM stock solution) or 10% DMSO; Component 5: 5 μl of ^{33}P-ATP (25 μM stock solution, 0.8 μCi).

The assay was initiated by the addition of ^{33}P-ATP and the reaction mixture incubated at ambient temperature for 20–40 minutes, depending on the protein kinase target. After the incubation period, the assay was terminated by spotting 10 μl of the reaction mixture onto a Millipore Multiscreen plate (Billerica, MA, USA). The Millipore Multiscreen plate was washed 3 times for approximately 15 minutes each in a 1% phosphoric acid solution. The radioactivity on the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter. Blank control, which

included all the assay components except the addition of the appropriate substrate (replaced with equal volume of assay dilution buffer), was set up for each protein kinase target. The corrected activity for each protein kinase target was determined by removing the blank control value. Activity of the 50 kinase targets in the presence of compound **2c** is shown in Figure 4.

REFERENCES

1. Gao, Z.-G.; Duong, H.T.; Sonina, T.; Kim, S.-K.; Van Rompaey, P.; et al. A. Orthogonal activation of the reengineered A3 adenosine receptor (neoceptor) using tailored nucleoside agonists. *J. Med. Chem.* **2006**, 49, 2689–2702.
2. Hong, C.I.; Chheda, G.B.; Dutta, S.P.; O'Grady-Curtis, A.; Tritsch, G.L. Synthesis and biological activity of analogs of naturally occurring 6-ureidopurines and their nucleosides. *J. Med. Chem.* **1973**, 16, 139–147.
3. Sigmund, H.; Pfleiderer, W. Nucleotides Part LXXI: A new type of labelling of nucleosides and nucleotides. *Helvetica Chim. Acta* **2003**, 86, 2299–2334.
4. Nakano, S.-I.; Uotani, Y.; Nakashima, S.; Anno, Y.; Fujii, M.; Sugimoto, N. Large stabilization of a DNA duplex by the deoxyadenosine derivatives tethering an aromatic hydrocarbon group. *J. Am. Chem. Soc.* **2003**, 125, 8086–8087.
5. Anzai, K.; Hunt, J.B.; Zon, G. Reactions of ethyl and phenyl chloroformate with adenosine derivatives as an entry to N6-ureido-linked spin-labeled adenosine and other modified adenosines. *J. Org. Chem.* **1982**, 47, 4281–4285.
6. Montgomery, J.A.; Thomas, H.J. Nitrosoureidonucleosides. *J. Med. Chem.* **1979**, 22, 1109–1113.
7. Peterson, M.A.; Ke, P.; Shi, H.; Jones, C.; McDougall, B.R.; Robinson, W.E. Design, synthesis, and antiviral evaluation of some 3′ -carboxymethyl-3′-deoxyadenosine derivatives. *Nucleos. Nucleotid. Nucl.* **2007**, 26, 499–519.
8. Robins, M.J.; Sarker, S.; Xie, M.; Zhang, W.; Peterson, M.A. Synthesis of 2′, 3′-fused (3.3.0)γ-butyrolactone-nucleosides and coupling with amino nucleosides to give amide-linked nucleotide-dimer analogues. *Tetrahedron Lett.* **1996**, 37, 3921–3924.
9. Peterson, M.A.; Nilsson, B.L.; Sarker, S.; Doboszewski, B.; Zhang, W.; Robins, M.J. Amide-lined ribonucleoside dimers derived from 5′-amino-5′deoxy- and 3′-(carboxymethyl)-3′-deoxynucleoside precursors. *J. Org. Chem.* **1999**, 64, 8183–8192.
10. Holbeck, S.L. Update on NCI in vitro drug screening utilities. *Eur. J. Cancer* **2004**, 40, 785–793.
11. Paull, K.D.; Shoemaker, R.H.; Hodes, L.; Monks, A.; Scudiero, D.A.; et al. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J. Natl. Cancer Inst.* **1989**, 81, 1088–92.
12. Ambit Biosciences. March, 2009 < http://www.ambitbio.com/>
13. Fabian, M.A.; Biggs, W.H. III; Treiber, D.K.; Atteridge, C.E.; Azimioara, M.D.; et al. A Small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotech.* **2005**, 23, 329–336.
14. Karaman, M.W.; Herrgard, S.; Treiber, D.K.; Gallant, P.; Atteridge, C.E., et al. A Quantitative analysis of kinase inhibitor selectivity. *Nature Biotech.* **2008**, 26, 127–132.
15. Helms, M.W.; Packeisen, J.; August, C.; Schittek, B.; Boecker, W.; Brandt, B.H.; Buerger, H. First evidence supporting a potential role for the BMP/SMAD pathway in the progression of oestrogen receptor-positive breast cancer. *J. Pathol.* **2005**, 206, 366–376.
16. Alarmo, E.-L.; Kuukasjärvi, T.; Karhu, R.; Kallioniemi, A. A comprehensive expression survey of bone morphogenetic proteins in breast cancer highlights the importance of BMP4 and BMP7. *Breast Cancer Res. Breast Cancer Res.* **2007**, 103, 239–246.
17. Anderson, G.J.; Darshan, D. Small-molecule dissection of BMP signaling. *Nature Chem. Biol.* **2008**, 4, 15–16.