Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 327–333
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

New bis-*N*9-(methylphenylmethyl)purine derivatives: Synthesis and antitumor activity

Nageswara Kode, Liying Chen, Devangachinta Murthy, Dare Adewumi, Shashikant Phadtare*

Xavier University of Louisiana, College of Pharmacy, Division of Basic Pharmaceutical Sciences, 1 Drexel Drive, New Orleans, LA 70125, USA

Received 5 April 2006; received in revised form 29 September 2006; accepted 26 October 2006
Available online 12 December 2006

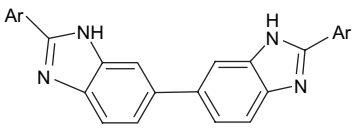
Abstract

A series of *ortho*-, *meta*- and *para*-bis-*N*9-(methylphenylmethyl)purine derivatives **4**–**15** were obtained by two-step synthesis from various substituted chloropurines with α,α′-dichloroxylenes. These bis-*N*9-(methylphenylmethyl)purines **4**–**15** were evaluated for the primary cytotoxic activity against a panel of NCI-H460 (lung), MCF-7 (breast) and SF-268 (CNS) cancer cell lines. The 'active' compounds which reduced growth of cancer cells to ca. 32% or less, have been evaluated in a full panel of 60 human cancer cell lines over a 5-log dose range at the National Cancer Institute, Bethesda, MD. In this series, the most activity is correlated to the compounds derived from the 2,6-dichloropurines such as bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**), bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**), and bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**). In particular compound **8** exhibited high sensitivity in leukemia cell lines and compounds **5**, **8** and **11** exhibited consistent high sensitivity in many breast cancer cell lines. Compound **11** was the most potent in this series and exhibited $GI_{50} < 0.01$ μM sensitivity against non-small lung cancer EKVX, colon cancer HT-29, melanoma SK-MEL-28, renal cancer RXF 393, prostate cancer DU-145 and several breast cancer HS 578T and BT-549 cell lines.

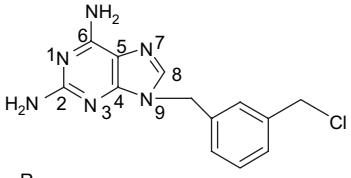
Keywords: Bisarylpurines; Bis-9-(methylphenylmethyl)chloropurines; Antitumor activity

1. Introduction

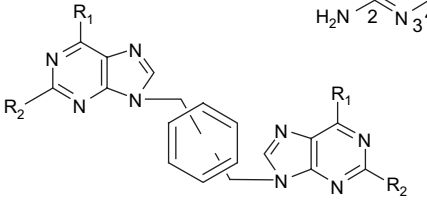
Several chemical classes of anticancer drugs have been identified through both empirical screening and rational design of new compounds during the past three decades [1]. These include several heterocyclic dimers such as bis-pyrrolobenzodiazepines [2], bis-alkylaminophenylfurans [3] and bis-benzimidazoles [4]. These heterocyclic dimers, with acylic and cyclic spacers, target the DNA to exhibit their anticancer activity by intercalation and alkylation mechanism [2,5]. Using these dimers as ''lead'' agents, both intercalating and irreversible alkylating dimers have been prepared to identify heterocycles that can induce DNA binding, interstrand cross-links and disrupt cellular processes necessary for cell maintenance and replication in cancer cells. Several bis-benzimidazole dimers such as **1**, with 'imidazole' heterocycle, have been reported to exhibit anticancer properties via DNA binding and interstrand cross-links in variety of cancer cell lines [4].



1



2



3

* Corresponding author. Tel.: +1 504 520 5378; fax: +1 504 520 7954.
E-mail address: sphadtar@xula.edu (S. Phadtare).

0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.10.017

As a part of our research program aimed at the design of new aromatic nucleic acid base derivatives as potential antitumor agents, we have been studying synthesis and biological evaluation of new arylpurine nucleosides such as **2** as potential antitumor agents [6]. In a previous paper, we reported the synthesis and anticancer activity of several 2,6-substituted chloromethyl arylpurines, related to **2**, with growth inhibitory effects (GI_{50}) in wide range of cancer cell lines at 10^{-5}–10^{-8} μM concentrations [7]. These results prompted us to design new purine nucleic acid 'heterocycle' based bis-9-(methylphenylmethyl)-purine dimers related to **3** as analogues of bis-benzimidazole dimer **1** as potential antitumor agents. Our rationale for designing new bis-9-(methylphenylmethyl)chloropurine derivatives related to **3** is based on the hypothesis that an appropriately substituted π-electron rich aromatic ring between a two 2,6-subsituted nucleic acid bases may provide improved DNA binding affinity, hydrogen bonding and sequence specificity compared to the nonnucleoside heterocyclic dimers and this may result, at least in part, in the therapeutic as well as toxic actions of the drug. In this paper, therefore, we describe the preparation and antitumor evaluation of a series of purine nucleic acid base *ortho*-, *meta*- and *para*-bis-9-(methylphenylmethyl)purine derivatives **4**–**15** as shown in Fig. 1.

2. Chemistry

Alkylation at both the ends of dichloro alkylating agent was achieved by the direct alkylation approach of commercially available purine bases such as 6-chloropurine, 2,6-dichloropurine or 2-amino-6-chloropurine with the α,α′-dichloro-*ortho*-xylene in the presence of base catalyst $CsCO_3$ in dimethylformamide (Scheme 1). A two-fold excess of purine base, 1 equiv of alkylating agent and 2 equiv of $CsCO_3$ base catalyst were employed to isolate bis-*N*9-*ortho*-methylarylmethylpurine dimers **4**–**5** as the major products in moderate to good yields. A small amount of *N*9-chlroromethyl derivatives related to **2** was also formed as the byproduct in addition to the dimers **4**–**5** [8]. Compound **4** was further aminated under pressure to the amine **6** in quantitative yield. Similar synthetic route was employed for the synthesis of bis-*N*9-*meta*-methylarylmethylpurines **7**–**9**, *N*9-*para*-methylarylmethylpurines **10**–**11** and *N*9-methylbiphenylpurines **12**–**15** starting with α,α′-dichloro-*m*-xylene and α,α′-dichloro-*p*-xylene, respectively. Alkylating agent 4,4′-bis(chloromethyl)-1,1′-biphenyl was employed in the synthesis of dimers **13**–**14**. Compounds **10** and **13** were further aminated under pressure to the amines **12** and **15** in quantitative yield, respectively. The structures of bisarylpurines **4**–**15** were confirmed by ^{1}H NMR and satisfactory elemental (C, H, N) analyses within ±0.4% of theoretical values.

3. Pharmacology

Evaluation of anticancer activity for the arylpurines **4**–**15**, described in this paper, was performed at the National Cancer Institute (NCI), Bethesda, MD. First arylpurines **4**–**15** were evaluated in the primary cytotoxic percent growth assay at 10^{-4} M concentration against a 3-cell line panel consisting of the NCI-H460 (lung), MCF-7 (breast), and SF-268 (CNS) cell lines (Table 1). In the assay protocol, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a 10^{-4} M concentration and incubated for 48 h. End point determinations were made with alamar blue. Results for the target compounds **4**–**15** were reported as the percent growth of the treated cells compared to untreated control cells.

For the NCI criteria, compounds which reduce the growth of any one of the cancer cell lines to ca. 32% or less are identified as 'active' compounds and are subsequently evaluated in a full panel of 60 cell lines over a 5-log dose range. The

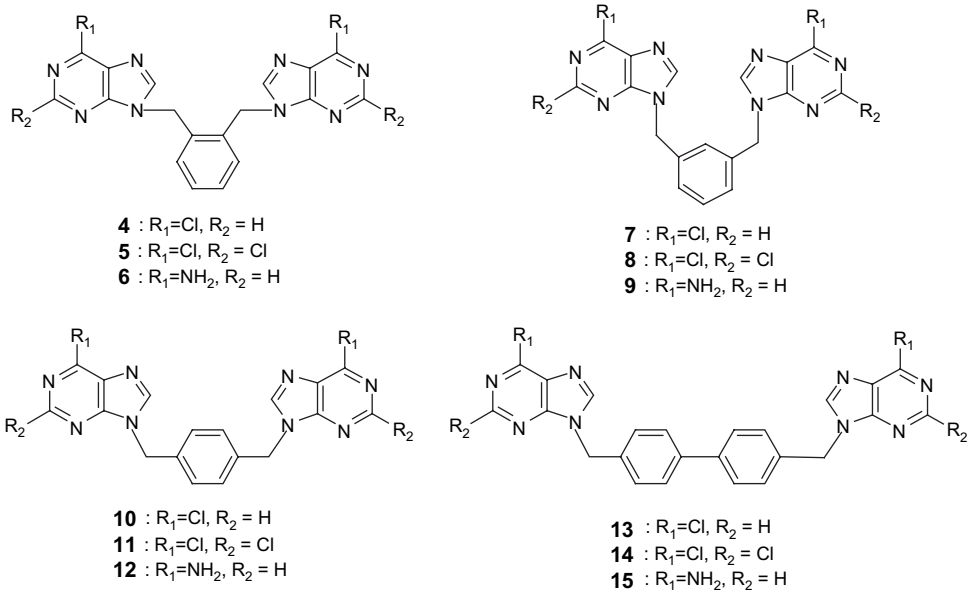
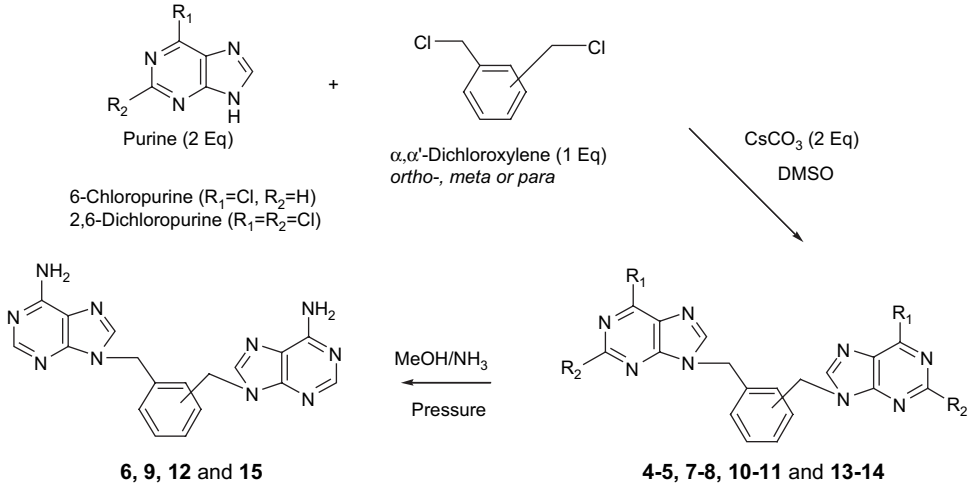


Fig. 1.



Scheme 1.

bis-*N*9-arylpurines derived from 2,6-dichloropurines such as bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**), bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**), and bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**) reached these criteria and were evaluated for their anticancer activity following the known in vitro disease oriented antitumor screening program which is based upon the use of multiple panel of 60 human tumor cell lines [9,10]. A 48 h continuous drug exposure protocol is used and a sulforhodamine B (SRB) protein assay is used to estimate cell viability or growth [11]. The anticancer activity of each compound is deduced from dose response curves and is presented in Table 2 according to the data provided by NCI [12]. The response parameters GI_{50}, TGI and LC_{50} refer to the drug concentration that produced 50% inhibition, total growth inhibition, and 50% cytotoxicity, respectively, and are expressed in micromolar (μM) concentrations.

4. Results and discussion

As shown in Table 1, the bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**), bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**), and bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**) exhibited <32% growth of breast (MCF-7), lung (NCI-H460) and/or CNS (SF-268) cancer cells at 100 μM concentrations and were identified as 'active' compounds and passed on to the evaluation in the full panel of 60 cancer cell lines over a 5-log dose range (0.01, 0.1, 1, 10, and 100 μM). The 60 panel cell evaluation data are presented in Table 2.

From the analysis of the data for the bis-9-(methylphenylmethyl)2,6-chloropurines **5**, **8** and **11** reported in Table 2, we can evince that all the compounds tested in 60 panel cell lines demonstrated high growth inhibition activity at micromolar concentrations in several cell lines. In particular, the bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**) exhibited high sensitivity against non-small lung cancer NCI-H522 (GI_{50} 3.39 μM) and several breast cancer MCF-7, HS 578T, T-47D (GI_{50} 1.55, 3.09, 2.19 μM) cell lines. The bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**) also exhibited high sensitivity against all leukemia CCRF-CEM, K562, MOLT-4, RPMI-8226 (GI_{50} 1.41, 1.95, 1.66 and 0.55 μM), colon cancer COLO 205 (GI_{50} 3.38 μM), and several breast cancer MCF-7, T-47D (GI_{50} 3.63, 3.02 μM) cell lines. The bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**) was the most potent in this series and exhibited $GI_{50} < 0.01$ μM sensitivity against non-small lung cancer EKVX, colon cancer HT-29, melanoma SK-MEL-28, renal cancer RXF 393, prostate cancer DU-145 and several breast cancer HS 578T and BT-549 cell lines. The cytotoxicity data (LC_{50}) indicate that the bisarylpurines **5**, **8** and **11** are cytotoxic in limited number of cell lines.

From the above results of *ortho*-, *meta*- and *para*-bis-9-(methylphenylmethyl)chloropurines **4**–**15** summarized in Tables 1 and 2, it is clear that the most activity in this series is correlated to compounds derived from 2,6-dichloropurines such as bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**), bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**), and bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**). In particular compound **8** exhibited high activity in leukemia

Table 1
Primary assay results for anticancer activity of bisarylpurines **4**–**15**

Compound	R_1	R_2	Growth percentage at 10^{-4} M concentration cancer cell line		
			NCI-H460 (lung)	MCF-7 (breast)	SF-268 (CNS)
4	Cl	H	58	78	78
5	Cl	Cl	0	0	0
6	NH_2	H	84	99	99
7	Cl	H	65	77	90
8	Cl	Cl	0	0	0
9	NH_2	H	96	109	108
10	Cl	H	95	101	86
11	Cl	Cl	0	0	58
12	NH_2	H	111	112	96
13	Cl	H	45	67	95
14	Cl	Cl	N.t.[a]	N.t.	N.t.
15	NH_2	H	98	86	50

[a] N.t. = not tested.

Table 2
GI_{50}, TGI and LC_{50} values (μM) of bisarylpurines **5**, **8** and **11** against different tumor cell lines

Panel/cell line	Compound								
	5			**8**			**11**		
	GI_{50}[a]	TGI[b]	LC_{50}[c]	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia									
CCRF-CEM	1.41	3.09	6.61	2.23	5.37	>100	10.23	22.90	51.28
K562	1.95	4.47	15.49	3.09	16.98	>100	2.88	16.59	40.73
MOLT-4	1.66	3.63	8.13	3.46	13.80	>100	7.94	20.89	45.70
RPMI-8226	0.55	2.34	N.d.[d]	2.57	11.22	>100	6.45	25.11	75.85
Non-small cell lung cancer									
A549/ATCC	4.79	20.42	>100	11.22	26.30	61.65	N.d.	25.11	50.11
EKVX	1.02	2.63	6.76	3.09	10.96	41.68	<0.01	19.95	44.66
HOP-62	21.88	39.81	72.44	5.01	17.78	51.28	N.d.	26.30	51.28
HOP-92	1.91	4.17	9.12	6.16	22.38	70.79	N.d.	26.31	57.54
NCI-H226	3.09	7.24	26.92	4.78	26.91	>100	16.98	39.81	93.32
NCI-H23	15.49	32.36	67.61	8.51	21.87	52.48	13.80	26.91	51.28
NCI-H322M	14.45	27.54	52.48	14.13	27.54	52.48	N.d.	33.88	91.20
NCI-H460	3.98	17.38	89.13	3.71	13.49	58.88	14.13	26.91	52.48
NCI-H522	3.39	9.12	>100	2.88	7.07	41.68	12.30	25.70	52.48
Colon cancer									
COLO 205	3.16	>100	>100	3.38	11.22	>100	N.d.	17.37	41.68
HCC-2998	–	4.90	21.38	2.39	7.07	25.70	N.d.	25.11	50.11
HCT-116	3.31	13.80	>100	2.45	6.30	22.38	9.33	21.38	46.77
HCT-15	1.86	4.47	12.59	5.75	22.38	74.13	13.18	25.70	51.28
HT-29	3.31	>100	>100	4.46	>100	>100	<0.01	15.84	39.81
KM12	3.31	10.00	34.67	2.88	8.91	33.11	14.13	27.54	52.48
SW-620	1.86	3.98	8.32	1.82	3.31	6.16	<0.01	2.63	8.31
CNS cancer									
SF-268	6.76	26.92	95.50	12.88	31.62	75.85	15.49	38.90	>100
SF-295	15.14	28.18	57.30	16.60	30.90	57.54	19.05	40.73	85.11
SF-539	0.01	0.14	0.49	1.38	3.16	7.07	N.d.	13.80	37.15
SNB-19	3.98	>100	>100	3.02	13.80	60.25	N.d.	21.87	46.77
SNB-75	5.13	21.88	81.28	5.62	22.38	93.32	<0.01	16.98	41.68
U251	1.78	3.55	69.18	3.89	13.80	38.90	13.80	26.91	51.28
Melanoma									
LOX IVIVI	1.15	2.75	6.61	2.23	5.62	21.87	2.88	17.37	41.68
M14	15.85	38.90	61.66	2.75	7.58	33.88	N.d.	27.54	52.48
SK-MEL-2	6.31	26.92	>100	2.81	7.76	35.48	N.d.	23.44	48.97
SK-MEL-28	3.98	24.55	>100	4.26	22.38	>100	<0.01	18.19	42.65
SK-MEL-5	2.24	6.17	22.91	2.45	5.88	19.05	10.72	22.90	47.86
UACC-257	7.94	23.44	61.66	4.78	17.37	56.23	N.d.	25.11	50.11
UACC-62	1.74	3.39	6.61	2.39	5.88	22.38	13.80	26.91	51.28
Ovarian cancer									
IGROV1	5.25	17.78	57.54	7.41	20.41	47.86	12.88	25.11	50.11
OVCAR-3	1.66	3.02	5.50	2.39	7.24	28.18	13.49	26.30	51.28
OVCAR-4	2.09	4.23	8.71	3.71	13.18	77.62	14.13	27.54	52.48
OVCAR-5	20.89	38.02	70.80	15.14	28.84	54.95	N.d.	26.30	51.28
OVCAR-8	3.02	8.13	48.98	4.67	18.62	72.44	10.72	22.38	47.86
SK-OV-3	11.48	31.62	87.10	N.t.[e]	N.t.	N.t.	N.t.	N.t.	N.t.
Renal cancer									
786-0	10.00	25.12	63.10	12.30	26.30	57.54	14.45	27.54	52.48
A498	16.98	33.88	67.61	4.57	17.37	46.77	N.d.	22.90	47.86
ACHN	1.77	3.24	5.75	2.51	6.16	21.37	12.59	25.11	50.11
CAKI-1	1.73	3.09	5.62	12.02	26.30	57.54	15.85	30.20	57.54
RXF 393	–	3.47	7.08	1.90	3.71	7.24	<0.01	0.010	20.89
SN12C	2.51	6.61	26.92	4.67	18.19	60.25	14.13	27.54	52.48
TK-10	2.69	7.94	28.84	2.45	7.24	26.91	13.18	26.30	51.28
UO-31	1.95	3.80	7.41	1.90	3.54	6.60	10.00	21.38	46.77
Prostate cancer									
PC-3	2.24	6.76	38.91	3.98	13.49	40.73	12.88	25.70	50.11
DU-145	1.00	2.51	6.46	1.73	3.09	5.62	<0.01	4.16	26.30

Table 2 (*continued*)

Panel/cell line	Compound								
	5			**8**			**11**		
	GI_{50}[a]	TGI[b]	LC_{50}[c]	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Breast cancer									
MCF-7	1.55	4.57	>100	3.63	37.15	>100	10.47	25.11	61.66
NCI/ADR-RES	20.42	43.65	91.20	12.59	34.67	97.72	13.80	28.84	60.25
MDA-MB-231/ATC	1.82	3.39	6.46	2.63	7.07	30.19	N.d.	21.87	46.77
HS 578T	3.09	11.22	>100	2.69	7.24	83.17	<0.01	16.21	43.65
MDA-MB-435	2.40	5.50	17.38	7.24	22.38	58.88	16.60	31.62	61.66
BT-549	0.17	1.51	5.01	3.38	10.96	34.67	<0.01	19.05	43.65
T-47D	2.19	5.89	>100	3.02	8.51	>100	1.62	13.49	42.65

[a] GI_{50} = concentration at which the percent growth is 50% (50% inhibition).
[b] TGI = concentration at which the total growth inhibition is 100%.
[c] LC_{50} = concentration at which the cytotoxicity is 50%.
[d] N.d. = not determined.
[e] N.t. = not tested.

cell lines and compounds **5**, **8**, **11** exhibited consistent high activity in many breast cancer cell lines. The bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine **11** was the most potent in this series and exhibited $GI_{50} < 0.01$ μM activity against non-small lung cancer EKVX, colon cancer HT-29, melanoma SK-MEL-28, renal cancer RXF 393, prostate cancer DU-145 and several breast cancer HS 578T and BT-549 cell lines These results clearly indicate that the presence of a π-electron rich ''aromatic spacer'' alone cannot be a sole determinant factor in the biological activity of bisarylpurines, but an appropriate chloropurine base such as 2,6-dichloropurine may add to the greater potential for the biological activity of the compounds described in this series.

5. Conclusions

In summary, the 2,6-dichloropurine agents in this series such as bis-9-[*o*-(methylphenylmethyl)]2,6-dichloropurine (**5**), bis-9-[*m*-(methylphenylmethyl)]2,6-dichloropurine (**8**), and bis-9-[*p*-(methylphenylmethyl)]2,6-dichloropurine (**11**) exhibited significant cytotoxic activity against several cancer cell lines. It is apparent from this study that the presence of an aromatic ring spacer between two 2,6-dicloropurine bases led to an antitumor aromatic nucleoside derivatives **5**, **8** and **11**. Antitumor activity of compound **14** is currently under investigation. To investigate this hypothesis further, we are currently studying the synthesis and biological evaluation of new bisarylpurine derivatives of 2,6-dichloropurine with pyridyl, biphenyl, naphthyl, quinoxyl and other carbocyclic spacers as potential anticancer agents.

6. Experimental protocol

6.1. Chemistry

All chemicals and reagents not otherwise noted were purchased from Sigma–Aldrich Chemical Co. Melting points were determined on a Haake–Buchler melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker 500 MHz and Anasazi Eft-90 MHz spectrometers. UV spectra were recorded on a Beckman–Coulter DU-800 spectrophotometer. Analytical TLC was carried out on Sigma–Aldrich 0.2 mm precoated silica gel polyester sheets with UV indicator. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Analysis of C, H, and N was within ±0.4% of theoretical values.

6.1.1. General procedure for the synthesis of bis-N9-arylpurines *4–5, 7–8, 10–11* and *13–14*

To a solution of purine base (20 mmol) and anhydrous $CsCO_3$ (20 mmol) in dry dimethylsulfoxide (DMSO, 30 ml), alkylating agents *ortho*-, *meta*-, *para*-dichloroxylene, or 4,4′-bis(chloromethyl)-1,1′-biphenyl (10 mmol) were added. The reaction mixture was stirred at room temperature and the progress of the reaction was followed by thin layer chromatography (TLC). The DMSO was evaporated in vacuo and the residue was purified on a silica gel column chromatography using a dichloromethane:methanol mixture to give compounds **4–5**, **7–8**, **10–11** and **13–14** in moderate yields.

6.1.2. Bis-9-[o-(methylphenylmethyl)]6-chloropurine (*4*)

Compound **4** was prepared in 22% yield by the protocol described in the general procedure starting from 6-chloropurine and α,α′-dichloro-*ortho*-xylene at room temperature for 3 h. M.p. 209–212 °C. UV (0.1 M phosphate buffer pH 7) max 265, 205 nm. ^{1}H NMR (DMSO-d_6) δ: 8.99 (2H, s, 2 × H-8), 8.98 (2H, s, 2 × H-2), 7.65–7.25 (4H, m, Ar), 6.08 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{12}N_8Cl_2$) Calc: C, 52.57; H, 2.93; N, 27.24; found: C, 52.64; H, 3.23; N, 27.51%.

6.1.3. Bis-9-[o-(methylphenylmethyl)]2,6-dichloropurine (*5*)

Compound **5** was prepared in 29% yield by the protocol described in the general procedure starting from 2,6-chloropurine and α,α′-dichloro-*ortho*-xylene at room temperature for 3 h. M.p. 235–238 °C. UV (0.1 M phosphate buffer pH 7)

max 275, 205 nm. ^{1}H NMR (DMSO-d_6) δ: 8.14 (2H, s, 2 × H-8), 6.95–6.65 (4H, m, Ar), 5.23 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{10}N_8Cl_4$) Calc: C, 45.02; H, 2.09; N, 23.33; found: C, 45.25; H, 2.21; N, 23.67%.

*6.1.4. Bis-9-[o-(methylphenylmethyl)]adenine (**6**)*

Compound **6** was prepared in 92% yield from bis-9-[*o*-(methylphenylmethyl)]6-chloropurine (**5**). Compound **5** (1 mmol) was dissolved in 15 ml ammonia saturated with anhydrous methanol in a pressure tube. The pressure tube was tightly sealed and the mixture was refluxed for 6 h. The pressure tube was cooled in an ice bath and precipitated compound was isolated and crystallized from methanol to afford compound **6** in 92% yield. M.p. 288 °C (decompose). UV (0.1 M phosphate buffer pH 7) max 265, 215 nm. ^{1}H NMR (DMSO-d_6) δ: 8.75 (2H, s, 2 × H-8), 8.44 (2H, s, 2 × H-2), 7.49 (8H, m, Ar + 2 × NH_2), 5.84 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{16}N_{10}$) Calc: C, 58.05; H, 4.33; N, 37.61; found: C, 58.44; H, 4.23; N, 37.58%.

*6.1.5. Bis-9-[m-(methylphenylmethyl)]6-chloropurine (**7**)*

Compound **7** was prepared in 39% yield by the protocol described in the general procedure starting from 6-chloropurine and α,α′-dichloro-*meta*-xylene at room temperature for 3 h. M.p. 215–216 °C. UV (0.1 M phosphate buffer pH 7) max 265, 205 nm. ^{1}H NMR (DMSO-d_6) δ: 8.71 (2H, s, 2 × H-8), 8.64 (2H, s, 2 × H-2), 7.25–7.20 (4H, m, Ar), 5.43 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{12}N_8Cl_2$) Calc: C, 52.57; H, 2.93; N, 27.24; found: C, 52.85; H, 3.15; N, 27.21%.

*6.1.6. Bis-9-[m-(methylphenylmethyl)]2,6-dichloropurine (**8**)*

Compound **8** was prepared in 25% yield by the protocol described in the general procedure starting from 2,6-chloropurine and α,α′-dichloro-*meta*-xylene at room temperature for 3 h. M.p. 175–177 °C. UV (0.1 M phosphate buffer pH 7) max 275, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.34 (2H, s, 2 × H-8), 6.88–6.70 (4H, m, Ar), 5.03 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{10}N_8Cl_4$) Calc: C, 45.02; H, 2.09; N, 23.33; found: C, 45.41; H, 2.22; N, 23.48%.

*6.1.7. Bis-9-[m-(methylphenylmethyl)]adenine (**9**)*

Compound **9** was prepared in 98% yield from bis-9-[*m*-(methylphenylmethyl)]6-chloropurine (**7**). Compound **7** (1 mmol) was dissolved in 15 ml ammonia saturated anhydrous methanol in a pressure tube. The pressure tube was tightly sealed and the mixture was refluxed for 6 h. The pressure tube was cooled in an ice bath and precipitated compound was isolated and crystallized from methanol to afford compound **6** in 98% yield. M.p. 275 °C (decompose). UV (0.1 M phosphate buffer pH 7) max 265, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 7.89 (2H, s, 2 × H-8), 7.80 (2H, s, 2 × H-2), 7.05 (4H, s, 2 × NH_2), 6.98–6.93 (4H, m, Ar), 5.03 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{16}N_{10}$) Calc: C, 58.05; H, 4.33; N, 37.61; found: C, 58.35; H, 4.35; N, 37.65%.

*6.1.8. Bis-9-[p-(methylphenylmethyl)]6-chloropurine (**10**)*

Compound **10** was prepared in 23% yield by the protocol described in the general procedure starting from 6-chloropurine and α,α′-dichloro-*para*-xylene at room temperature for 3 h. M.p. 240–242 °C. UV (0.1 M phosphate buffer pH 7) max 265, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.81 (2H, s, 2 × H-8), 8.75 (2H, s, 2 × H-2), 7.33 (4H, s, Ar), 5.50 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{12}N_8Cl_2$) Calc: C, 52.57; H, 2.93; N, 27.24; found: C, 52.44; H, 2.98; N, 27.31%.

*6.1.9. Bis-9-[p-(methylphenylmethyl)]2,6-dichloropurine (**11**)*

Compound **11** was prepared in 25% yield by the protocol described in the general procedure starting from 2,6-chloropurine and α,α′-dichloro-*para*-xylene at room temperature for 3 h. M.p. 265–267 °C. UV (0.1 M phosphate buffer pH 7) max 275, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.78 (2H, s, 2 × H-8), 7.30 (4H, s, Ar), 5.45 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{10}N_8Cl_4$) Calc: C, 45.02; H, 2.09; N, 23.33; found: C, 45.09; H, 2.39; N, 23.51%.

*6.1.10. Bis-9-[p-(methylphenylmethyl)]adenine (**12**)*

Compound **12** was prepared in 98% yield from bis-9-[*p*-(methylphenylmethyl)]6-chloropurine (**10**). Compound **10** (1 mmol) was dissolved in 15 ml ammonia saturated anhydrous methanol in a pressure tube. The pressure tube was tightly sealed and the mixture was refluxed for 6 h. The pressure tube was cooled in an ice bath and precipitated compound was isolated and crystallized from methanol to afford compound **6** in 92% yield. M.p. 290 °C (decompose). UV (0.1 M phosphate buffer pH 7) max 265, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.20 (2H, s, 2 × H-8), 8.11 (2H, s, 2 × H-2), 7.26 (4H, s, Ar), 7.20 (4H, s, 2 × NH_2), 5.32 (4H, s, 2 × CH_2N). Anal. ($C_{18}H_{16}N_{10}$) Calc: C, 58.05; H, 4.33; N, 37.61; found: C, 58.14; H, 4.50; N, 37.75%.

*6.1.11. Bis-9-[4,4′-bis(methyl)-1,1′-biphenyl]6-chloropurine (**13**)*

Compound **13** was prepared in 32% yield by the protocol described in the general procedure starting from 6-chloropurine and 4,4′-bis(chloromethyl)-1,1′-biphenyl at room temperature for 3 h. M.p. 216–217 °C. UV (0.1 M phosphate buffer pH 7) max 265, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.87 (2H, s, 2 × H-8), 8.79 (2H, s, 2 × H-2), 7.61–7.59 (4H, m, Ar), 7.43–7.42 (4H, m, Ar), 5.56 (4H, s, 2 × CH_2N). Anal. ($C_{24}H_{16}N_8Cl_2$) Calc: C, 59.14; H, 3.30; N, 22.99; found: C, 59.52; H, 3.41; N, 23.01%.

*6.1.12. Bis-9-[4,4′-bis(methyl)-1,1′-biphenyl]2,6-dichloropurine (**14**)*

Compound **14** was prepared in 25% yield by the protocol described in the general procedure starting from 2,6-chloropurine and 4,4′-bis(chloromethyl)-1,1′-biphenyl at room temperature for 3 h. M.p. 275–276 °C. UV (0.1 M phosphate buffer pH 7) max 270, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 9.25 (2H, s, 2 × H-8), 8.08–7.74 (8H, m, Ar), 5.91 (4H, s, 2 × CH_2N). Anal. ($C_{24}H_{14}N_8Cl_4$) Calc: C, 51.82; H, 2.53; N, 20.14; found: C, 52.02; H, 2.23; N, 20.22%.

*6.1.13. Bis-9-[4,4′-bis(methyl)-1,1′-biphenyl]adenine (**15**)*

Compound **15** was prepared in 98% yield from bis-9-[4,4′-bis(methyl)-1,1′-biphenyl]6-chloropurine (**13**). Compound **13** (1 mmol) was dissolved in 15 ml ammonia saturated anhydrous methanol in a pressure tube. The pressure tube was tightly sealed and the mixture was refluxed for 6 h. The pressure tube was cooled in an ice bath and precipitated compound was isolated and crystallized from methanol to afford compound **6** in 98% yield. M.p. 292 °C (decompose). UV (0.1 M phosphate buffer pH 7) max 265, 210 nm. ^{1}H NMR (DMSO-d_6) δ: 8.27 (2H, s, 2 × H-8), 8.14 (2H, s, 2 × H-2), 7.59–7.58 (4H, m, Ar), 7.38–7.37 (4H, m, Ar), 7.22 (4H, s, 2 × NH_2), 5.39 (4H, s, 2 × CH_2N). Anal. ($C_{24}H_{20}N_{10}$) Calc: C, 64.27; H, 4.49; N, 31.23; found: C, 64.36; H, 4.58; N, 31.33%.

6.2. Pharmacology

The compounds were tested by NCI in an in vitro 3-cell line, one dose primary anticancer assay as a primary cancer screen. The 3-cell line panel consists of the NCI-H460 (lung), MCF-7 (breast) and SF-268 (CNS). Each cell line was inoculated and preincubated on a microtriter plate. Test agents were then added at a single 10^{-4} M concentration and the culture incubated for 48 h. End point determinations were made with alamar blue [13]. Results from each test agent were reported as the percent growth of treated cells when compared with untreated control cells. Compounds which reduced the growth of any one of the cell lines to ca. 32% or less were passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

A total of 60 human cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, breast) formed the basis of this test. The tumor cells were cultured in RPMI1640 medium supplemented with 5% fetal calf serum and 2 mM L-glutamine. The tumor cells were inoculated over a series of standard 96-well microtrite plates in 100 μM of medium [9,10]. Density of inoculum depends on the type of tumor cell and its growth characteristics [14]. These cells were then preincubated on the microtiter plate for 24 h before adding the compounds. These were tested in DMSO solution at five different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M). After incubation of the chemical agent for 48 h with the tumor cell lines, a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cytotoxic effects were evaluated and the assay results and dose response parameters calculated as previously described [12].

Acknowledgments

We thank the Antitumor Evaluation Branch and Biological testing Branch of the National Cancer Institute, Bethesda, MD for the biological evaluation of the compounds described in this paper. We also thank the Tulane University Central Instrumentation Facility for the support of high resolution NMR. The work described herein was supported by a MBRS grant S06 GM008008-33 from DHHS/NIH/NIGMS.

References

[1] G. Schwartsmann, M.J. Ratain, G.M. Cragg, J.E. Wong, N. Saijo, D.R. Parkinson, Y. Fujiwara, R. Pazdur, D.J. Newman, R. Dagher, L. DiLeone, J. Clin. Oncol. 20 (2002) 47–59.
[2] S. Gregson, P. Howard, D. Gullick, A. Hamaguchi, K. Corcoran, N. Brooks, J. Hartley, T. Jenkins, S. Patel, M. Guille, J. Med. Chem. 47 (2004) 1161–1174.
[3] P.L. Bischoff, V. Holl, D. Coelho, P. Dufour, D. Weltin, B. Luu, Curr. Med. Chem. 7 (2000) 693–713.
[4] J. Mann, A. Baron, Y. Opoku-Boahen, E. Johansson, G. Parkinson, L.R. Kelland, S. Neidle, J. Med. Chem. 44 (2001) 138–144.
[5] S. Gregson, P. Howard, J. Hartley, N. Brooks, L. Adams, T. Jenkins, L. Kelland, D. Thurston, J. Med. Chem. 44 (2001) 737–748.
[6] M. Brakta, D. Murthy, L. Ellis, S. Phadtare, Bioorg. Med. Chem. Lett. 12 (2002) 1489–1492.
[7] D. Murthy, M. Brakta, L. Ellis, O. Abanah, S. Phadtare, Med. Chem. Res. 12 (2003) 13–25.
[8] S. Phadtare, D. Kessel, T.H. Corbett, H.E. Renis, B.A. Court, J. Zemlicka, J. Med. Chem. 34 (1991) 421–429.
[9] M.R. Grever, S.A. Schepartz, B.A. Chabner, Semin. Oncol. 19 (1992) 622–638.
[10] M.R. Boyd, K.D. Paul, Drug Dev. Res. 34 (1995) 91.
[11] P. Skehan, R. Storeng, D. Scudiero, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[12] M.R. Boyd, R.H. Shomaker, T.L. McLemore, New drug development, in: J.A. Roth, J.C. Ruckdeschel, T.H. Weisenburger (Eds.), Thoracic Oncology, Saunders, Philadelphia, 1989, pp. 711–721.
[13] G.D. Gray, E. Wickstorm, BioTechniques 21 (1996) 780–782.
[14] A. Monks, D. Scudiero, P. Skehan, R. Showmaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolf, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, J. Natl. Cancer Inst. 83 (1991) 757–766.