

Article

Synthesis and Cytotoxic Activity of Some New 2,6-Substituted Purines

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Abstract: A series of twenty four acyclic unsaturated 2,6-substituted purines **5a–20b** were synthesized. These compounds were evaluated for cytotoxic activity against NCI-60 DTP human tumor cell line screen at 10 μ M concentration. N₉-[(Z)-4'-chloro-2'-butenyl-1'-yl]-2,6-dichloropurine (**5a**), N₉-[4'-chloro-2'-butynyl-1'-yl]-2,6-dichloropurine (**10a**), N₉-[(E)-2',3'-dibromo-4'-chloro-2'-butenyl-1'-yl]-6-methoxypurine (**14**) and N₉-[4'-chloro-2'-butynyl-1'-yl]-6-(4-methoxyphenyl)-purine (**19**) exhibited highly potent cytotoxic activity with GI₅₀ values in the 1–5 μ M range for most human tumor cell lines. Other compounds exhibited moderate activity.

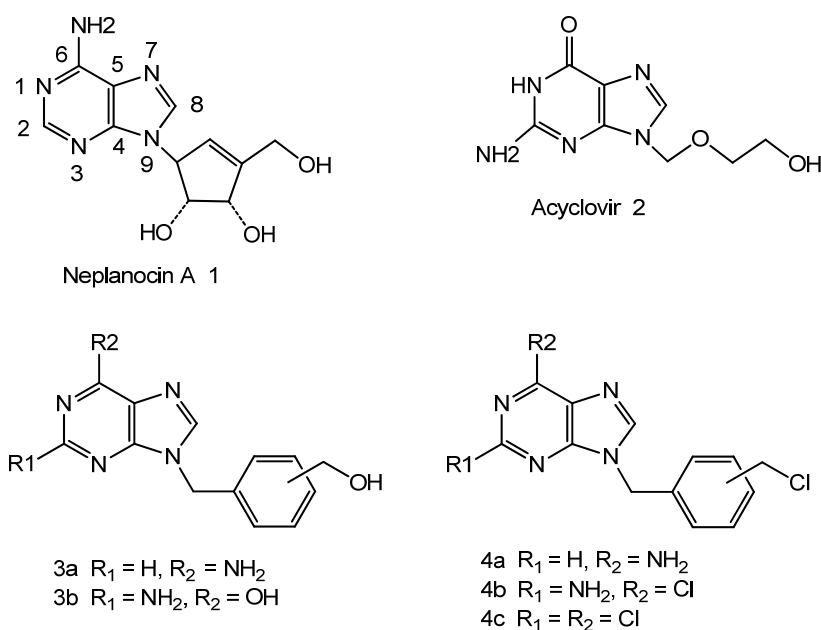
Keywords: Suzuki-Miyaura cross coupling reaction; pyridiniumtribromide bromination; 2,6-substituted purines; cytotoxic activity

1. Introduction

According to WHO report on cancer about 7.6 million people died in the year 2005 and the number is expected to raise to 9 million by the year 2015 and 11.5 million by 2030 [1]. Hence, development of new potent and selective anticancer agents has become one of most intensely pursued goals in drug development around the world. Neplanocin A, (**1**, Figure 1) is considered a carbocyclic analogue of a natural nucleoside and has shown potent antitumor and antiviral properties [2–4]. As a part of our research program on the synthesis of anti-cancer agents, we have synthesized some aromatic neplanocin-A analogues like **3a–3b**, **4a–c** [5–7].

The N₉-hydroxymethyl analogues of adenine, guanine and 2,6-diaminopurine related to **3a-3b** did not exhibit any anticancer activity, however, their N₉-chloromethyl arylpurine intermediates, related to **4a-4c** (Figure 1), were found to be potent *in vitro* growth inhibitors of several human tumor cell lines. These results prompted us to consider purines with an unsaturated N₉-linker that has been terminated with a chloromethyl group.

Figure 1. Neplanocin A and aromatic neplanocin-A analogues.



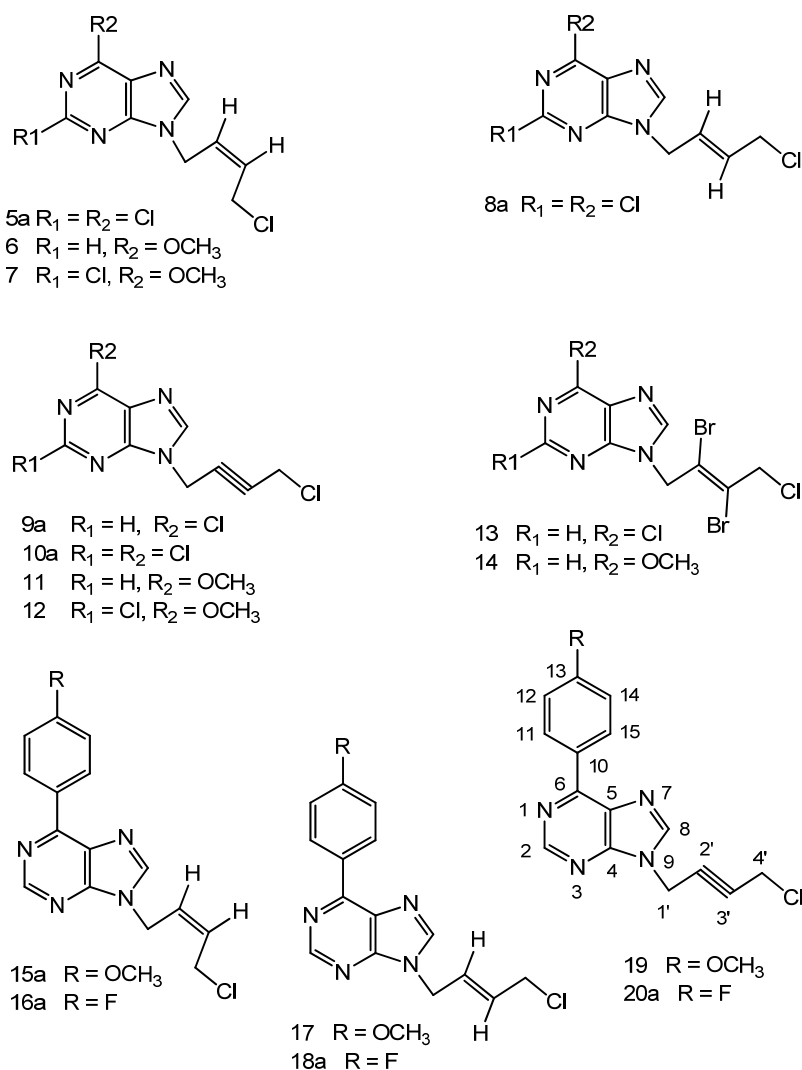
In the synthesis of anti-cancer purine compounds, many times either the purine base is modified or the sugar moiety is modified or replaced with a non-sugar linker or sometimes all these changes have been done by researchers simultaneously in an attempt to make an active compound. We have followed a very similar path in the present work.

Purine base selection: 2,6-Dichloropurine is selected where the chlorines are expected to serve as powerful electron withdrawing centers on the purine ring. 2-Chloro-6-methoxypurine is expected to serve a double role, with electron withdrawing and electron donating centers on the purine ring. The 6-methoxy group was selected for its electron donating nature to the purine ring. 6-(4-Methoxy)phenyl- and 6-(4-fluoro)phenyl-substituted purines were selected to significantly alter the purine base properties and to improve the lipophilicity. It is interesting to note that purines with those substitution patterns were also reported to elicit wide range of anti-viral and anti-cancer activities. 6-Methoxypurine arabinoside was reported as a potent inhibitor of Varicella-Zoster virus [8]. 6-Methoxy group-containing Nelarabine and the 2-chloro group-containing compound Clofarabine elicit anti-cancer activities [9]. Further, 6-(4-methoxyphenyl)purine and 6-(4-fluorophenyl)purine ribonucleosides were reported to elicit significant cytostatic activity [10].

Linker selection: We chose linkers like *cis*-1,4-dichlorobutene, *trans*-1,4-dichlorobutene and 1,4-dichlorobutene. All these linkers are acyclic five carbon length open chain linkers analogous to the linker of acyclovir, with some degree of unsaturation. All these linkers are common for each of the above purine bases selected, like 2,6-dichloropurine, 6-methoxypurine, 2-chloro-6-methoxypurine, 6-(4-methoxyphenyl) purine and 6-(4-fluorophenyl)purine. Reaction of each of these purine bases with

cis-a 1,4-dichlorobutene linker furnishes N₉ substituted purines with methylchloromethyl-*cis*-butene units, e.g., compounds **5a**, **6**, **7**, **15a** and **16a**. Reaction with the *trans*-1,4-dichlorobutene linker furnishes N₉ substituted purines with methylchloromethyl-*trans*-butene units, e.g., compounds **8a**, **17** and **18a**. Reaction with 1,4-dichlorobutyne is expected to furnish N₉ substituted purines with methylchloro-methyl-butyne moieties, e.g. compounds **9a**, **10a**, **11**, **12**, **19** and **20a**. Compounds **13** and **14** represent vinylic dibromides, a new class of purines, which were also synthesized in this work to assess their cytotoxic activity. This plan gives an opportunity for us to assess the cytotoxicity for a group of purine compounds (Figure 2) and to understand how the activity is changing for a given linker with a change on the substitution pattern on the purine ring.

Figure 2. Purines with acyclic unsaturated linkers.



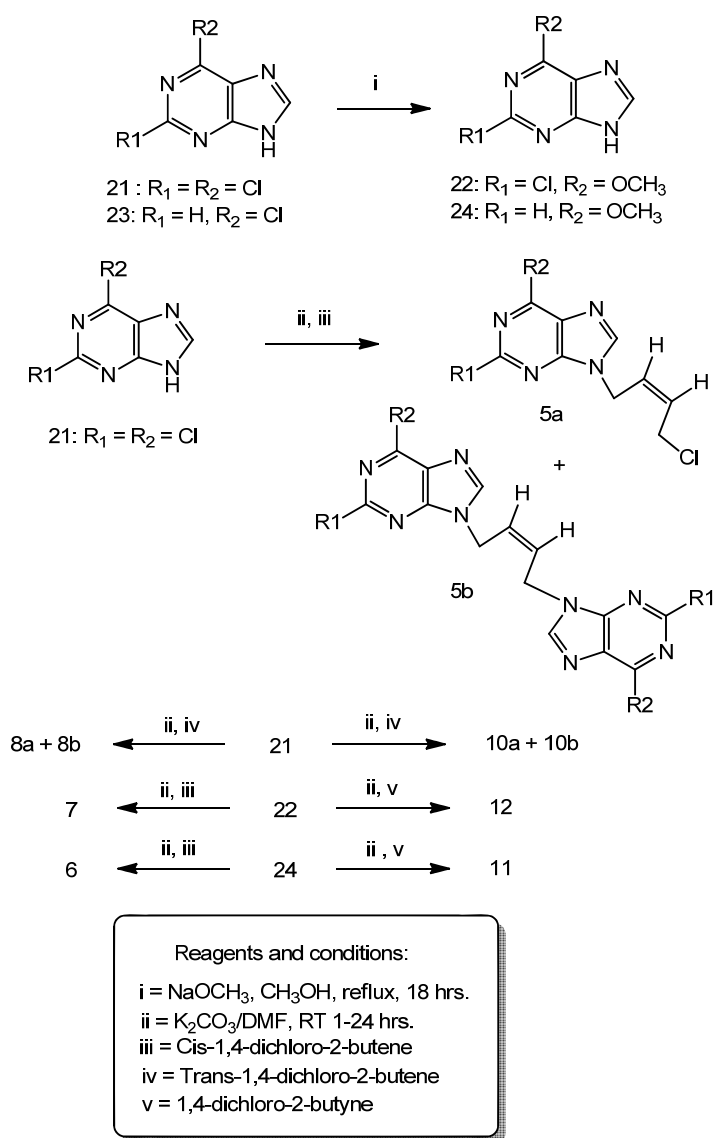
Here, we have focused primarily on the synthesis of N₉ substituted purines because they were found to be more active when compared to N₇ isomers at our laboratory. Furthermore the N₇ isomers are expected to be minor products in the synthesis.

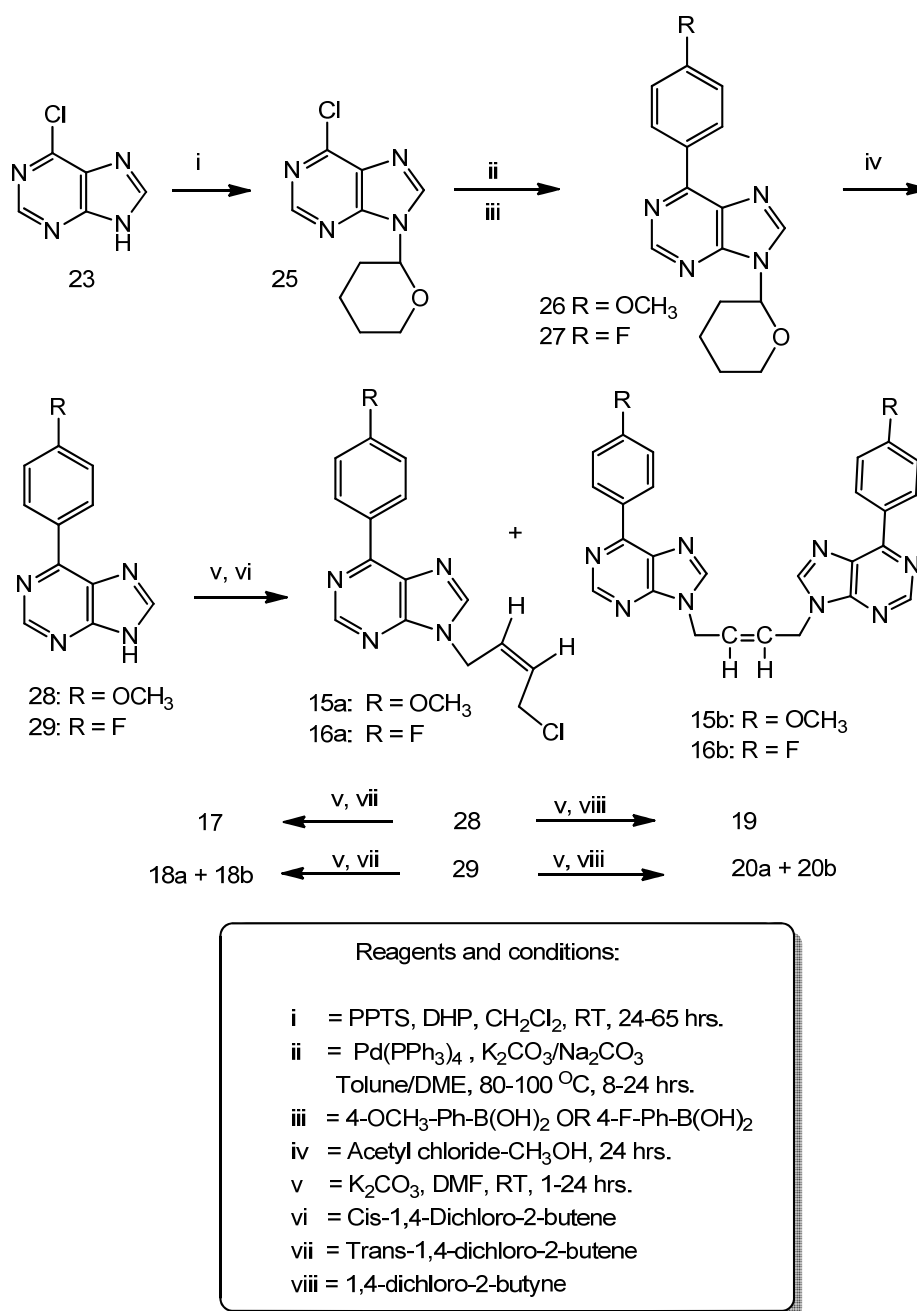
2. Results and Discussion

2.1. Chemistry

The N₉-alkylated compounds **5a-20b** were prepared by the direct alkylation approach on the appropriately substituted purine bases in presence of K₂CO₃ in dimethyl formamide (DMF) medium (Schemes 1 and 2). A 1-3 fold excess of the alkylating agent and anhydrous potassium carbonate were employed for one equivalent of the purine base taken to isolate N₉-purine isomers as the major product in moderate to good yields.

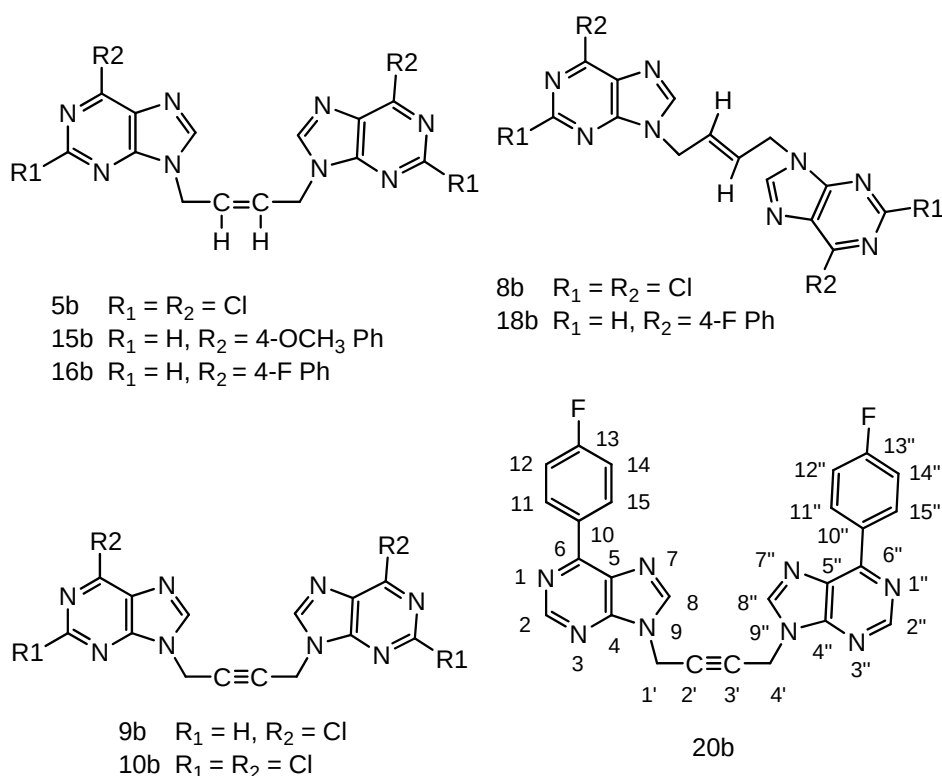
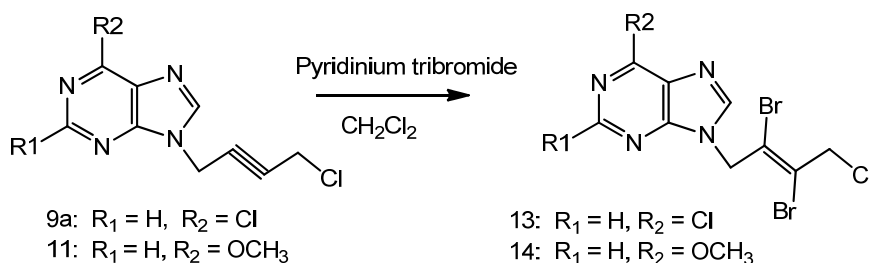
Scheme 1. Synthesis of N₉-alkylated compounds.



Scheme 2. Synthesis of 6-(4'-methoxyphenyl) and 6-(4'-fluorophenyl)-purines.

A ten equivalent excess is not required as previously reported [12]. Minor dimeric products (Figure 3) have been isolated whenever formed during the synthesis for each linker. Increasing the reaction time and the molar ratio of the potassium carbonate favors the higher yields of the dimeric products. The UV maxima for N7 isomers were 10–15 nm higher (275–320 nm) than the N₉-isomers (265–310 nm) [13].

Minor modifications were made to the Suzuki-Miyaura cross coupling procedure [10,14]. Reaction of appropriate phenylboronic acid with 9-(tetrahydropyran-2-yl)-6-chloropurine under Suzuki-Miyaura cross coupling methodology afforded 6-(4'-methoxyphenyl), 6-(4'-fluorophenyl)-purines (Scheme 2). The reported procedure of pyridiniumtribromide bromination of the acetylenic compounds [11] was adopted with minor modifications to furnish the vinylicdibromides **13**, **14** (scheme 3).

Figure 3. Dimers.**Scheme 3.** Synthesis of the vinylicdibromides **13**, **14**.

Acetyl chloride-mediated THP protection and deprotection of hydroxyl functional groups on a wide range of aliphatic and aromatic systems has been reported [15]. We have extended the concept to 6-chloropurine and found acetyl chloride to be a versatile clean inexpensive deprotective agent in methanol medium for the THP removal. No side products are found on the procedure we described, although Dowex 50W X 8 works [10]. During the protection of the NH of 6-chloropurine, acetyl chloride was found to form one side product, possibly an N-acetyl derivative, still the % yield was about 70 after column purification. The pyridinium-*p*-toluenesulfonate (PPTS) catalyzed THP protection of 6-chloropurine was found to be relatively very clean, no major side products formed and the yield was about 90%. Hence we chose PPTS catalyst for the THP protection of 6-chloropurine.

Reaction of sodium methoxide with 6-chloropurine and 2,6-dichloropurine in methanol medium furnished 6-methoxypurine and 2-chloro-6-methoxypurine respectively. Further reaction of these purine bases with various alkenyl and alkynyl linkers as explained above furnished the target compounds **5a-20b**. The structures of all the purines **5a-20b** were confirmed by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, LC-MS and satisfactory elemental (C, H, N) analysis within $\pm 0.4\%$ of theoretical values.

2.2. Cytotoxicity

Compounds **5a**, **5b**, **6**, **7**, **8a**, **8b**, **9a**, **9b**, **10a**, **10b**, **11**, **12**, **13**, **14**, **15a**, **15b**, **16a**, **16b**, **17**, **19**, **20** and **20b** were initially tested at 10 μ M concentration (Table 1).

Table 1. Cytotoxicity Data.

Cell Line	5a	5b	8b	10a	10b	14	17	19
Leukemia								
CCRF-CEM	−45	−73	18	−76	−62	−39	63	19
HL-60(TB)	−71	−70	−3.0	−70	−58	47	65	49
K-562	−63	−25	17	−67	−13	8	67	58
MOLT-4	−69	−55	81	−63	1.0	19	--	5.0
RPMI-8226	−53	−61	−17	−76	−57	−25	52	35
SR	−76	−48	−14	−76	−53	8.0	--	--
Non-Small Cell Lung Cancer								
A549/ATCC	−76	−7.0	72	−77	9.0	9.0	81	60
EKVX	−91	−68	87	−72	−39	114	108	115
HOP-62	−72	23	94	−82	62	16	99	77
HOP-92	−93	−34	−13	−92	−88	--	38	9.0
NCI-H226	−74	−59	94	−81	6.0	--	89	57
NCI-H23	−78	9.0	95	−70	39	15	64	59
NCI-H322M	−97	21	95	−100	47	79	100	92
NCI-H460	−63	4.0	84	−66	13	−41	67	68
NCI-H522	−85	−77	28	−89	−71	−72	49	49
Colon Cancer								
COLO 205	−65	−41	63	−77	−27	−82	87	80
HCC-2998	−87	--	--	−85	--	90	91	85
HCT-116	−80	−71	33	−100	−35	−82	46	52
HCT-15	−79	−44	50	−90	−66	32	61	50
HT-29	−61	−66	48	−70	−44	−57	85	94
KM-12	−86	−69	79	−73	−45	−6	85	73
SW-620	−87	−52	45	−89	−45	−74	61	49
CNS Cancer								
SF-268	−83	−2.0	62	−74	−38	12	88	63
SF-295	−92	−33	60	−84	6.0	97	109	95
SF-539	−90	−53	47	−93	−66	12	76	92
SNB-19	−89	10	75	−99	12	90	88	72
SNB-75	−92	−26	65	−97	8.0	47	128	94
U251	−72	−56	57	−96	−76	−72	64	29
Melanoma								
LOX IMVI	−83	−80	34	−89	−83	−87	54	−32
MALME-3M	−73	−72	111	−70	−75	−7	62	20
M14	−85	−33	101	−93	8.0	−25	71	70
MDA-MB-435	−90	−67	98	−86	4.0	4	58	40
SK-MEL-2	−77	24	96	−76	65	52	69	53
SK-MEL-28	−96	−19	99	−98	−26		98	87

Table 1. Cont.

SK-MEL-5	−91	−82	69	−96	−67	54	86	62
UACC-257	−90	--	--	−91	--	−41	81	59
UACC-62	−96	−73	79	−92	−12	57	77	51
Ovarian cancer								
IGROV1	−96	−82	18	−93	−75	12	94	46
OVCAR-3	−97	−99	61	−93	−91	−94	78	53
OVCAR-4	−86	−89	95	−88	−71	−47	53	51
OVCAR-5	−85	−9.0	112	−86	−3.0	62	114	97
OVCAR-8	−83	−57	8.0	−84	−35	1	67	65
NCI/ADR-Res	−56	30	99	−57	60	−40	77	56
SK-OV-3	−98	33	103	−99	88	148	111	86
Renal cancer								
786-0	−92	−9.0	93	−99	8.0	8.0	70	36
A498	−92	−18	90	−89	--	95	68	56
ACHN	−89	−94	90	−99	−53	15	59	53
CAKI-1	−95	−97	−60	−88	−96	96	71	62
RXF 393	--	--	--	--	3.0	70	8.0	30
SN12C	−84	−11	96	−87	--	11	84	46
TK-10	−86	−45	80	−93	−32	51	86	70
UO-31	−95	--	6.0	−98	--	57	60	−87
Prostate cancer								
PC-3	−93	−58	67	−98	−72	46	84	57
DU-145	−99	−98	44	−100	−87	37	82	88
Breast cancer								
MCF-7	−49	−78	38	−52	−23	0.0	40	36
MDA-MB-231/ATCC	−90	−68	96	−89	−53	42	89	70
HS578T	−43	−42	46	−44	--	24	131	107
BT549	−94	--	−33	−93	--	70	54	50
T-47D	−54	−44	38	−62	−21	1.0	68	53
MDA-MB-468	−72	--		−61	--	17	−4.0	21

* Where the number 100 = control growth, 0 = 100% inhibition, −100 = total cell kill. Compounds **5**, **6**, **7a**, **10**, **11**, **14a-b**, **15a-b**, **17a-b**, **19a-b** were not active at 10 μ M concentration. * Growth % for one dose testing at 10 μ M concentration.

Out of these compounds **6**, **7**, **8a**, **11**, **12**, **15a**, **15b**, **16a**, **16b**, **20a**, **20b** exhibited growth % in the range of 70–100 plus and hence may be considered inactive. Table 1 summarizes the single dose 10 μ M test results for the active compounds. Compounds **5a**, **5b**, **8b**, **10a**, **10b** and **14** elicited significant cytotoxicity on almost all the cell lines such as leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. Under the same single dose testing, compound **17** elicited cytotoxicity to MDA-MB-468 breast cancer cell line while compound **19** elicited cytotoxicity to LOXIMVI melanoma and UO-32 renal cancer cell lines. Table 2 represent the five dose testing results GI_{50} and LC_{50} for compounds **5a**, **10a**, **14** and **19**. Compound **5a** was found very active with GI_{50} values 1–2 μ M for almost all the cell lines. Compound **10a** was also found very active with GI_{50} values under 2 μ M for many cell lines. For leukemia HL-60TB and melanoma UACC-62 the GI_{50} values are 3.5 and 3.7 μ M respectively. Compound **14**

displayed impressive activity, with GI_{50} values of 2–3 μM for leukemia, melanoma, renal cancer and breast cancer. It also elicited significant activity on non-small cell lung cancer, colon cancer and CNS cancer. Compound **19** exhibited striking activity, with GI_{50} values of 2–4 μM for breast cancer and 2–8 μM for leukemia, colon, renal and prostate cell lines. Replacing the chlorine at 6-position in compound **5a** with a methoxy group results in compound **11**. Similarly replacing 6-chlorine in compound **5a** with a methoxy group results in compound **12**. These changes resulted in a total loss of cytotoxicity. When the N-9 *cis*-butene stereochemistry in **5a** is changed to a *trans* form as in **8a** also resulted in the loss of activity, although the corresponding dimer **8b** with a *trans* stereochemistry elicited good cytotoxicity.

On the 6-phenyl substituted compounds, only **19** elicited good activity and all other compounds were inactive. The reported procedure [11] was employed to transform the triple bond compounds **9a** and **11** in to the corresponding vinylic dibromides **13** and **14** (Scheme 2). Indeed one of the vinylic dibromide with a 6-methoxy substituent, **14**, was found very active for leukemia, melanoma, renal cancer, breast cancer (GI_{50} value 2–3 μM) and significant activity on all other cancers. The other vinylic dibromide with a 6-chlorine group **13** did not elicit any cytotoxicity. Compounds **18a** and **18b** were not tested.

Table 2. GI_{50} and LC_{50} data. Units: μM .

Compound	5a		10a		14		19	
Cell Line	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia								
CCRF-CEM	0.19	0.78	0.21	0.93	2.73	>100	3.84	>100
HL-60(TB)	0.33	>100	3.50	4.89	2.03	>100	3.06	>100
K-562	0.23	>100	0.22	4.89	3.29	>100	8.06	>100
MOLT-4	0.23	>100	0.50	>100	2.91	>100	2.68	>100
RPMI-8226	0.64	>100	0.23	>100	3.19	>100	3.46	>100
SR	--	--	--	--	2.73	>100	5.24	>100
Non-Small Cell Lung Cancer								
A549/ATCC	0.37	>100	0.54	>100	10.80	62.50	7.40	>100
EKVX	1.54	7.18	2.01	8.83	16.30	55.10	12.80	68.70
HOP-62	0.19	7.18	0.23	0.94	3.90	37.50	1.79	61.10
HOP-92	0.20	3.41	0.73	5.70	4.08	45.30	2.75	49.80
NCI-H226	1.30	5.10	1.61	5.70	2.82	30.70	4.45	>100
NCI-H23	0.22	5.10	0.19	0.84	3.16	39.40	7.83	50.50
NCI-H322M	1.64	5.68	1.63	5.59	12.40	50.20	12.50	52.30
NCI-H460	0.20	0.67	0.19	0.78	--	--	3.27	45.50
NCI-H522	1.01	7.51	2.41	29.0	0.88	5.27	3.09	55.10
Colon Cancer								
COLO 205	0.20	0.69	0.19	0.67	1.73	5.83	3.51	41.60
HCC-2998	0.20	0.71	0.27	0.72	17.20	56.90	17.60	57.20
HCT-116	0.18	0.71	0.20	0.76	1.73	7.05	3.50	41.30
HCT-15	0.17	0.65	0.18	0.73	1.84	9.39	4.06	54.90
HT-29	0.26	>100	0.23	>100	1.63	8.41	7.12	71.70
KM-12	0.22	0.80	0.36	3.73	3.54	37.60	4.84	45.20
SW-620	0.20	0.73	0.22	3.73	--	--	2.99	42.40

Table 2. Cont.

CNS Cancer								
SF-268	0.20	0.62	0.19	0.67	1.96	20.30	1.66	10.90
SF-295	1.29	6.97	1.60	0.70	10.60	51.60	2.35	40.30
SF-539	0.12	0.53	1.03	4.92	2.17	28.00	6.48	52.70
SNB-19	0.91	4.72	0.57	4.46	12.10	49.40	4.87	44.20
SNB-75	0.18	0.68	0.20	0.73	2.01	29.60	1.64	18.20
U251	0.18	0.57	0.18	0.62	1.74	7.62	1.96	>100
Melanoma								
LOX IMVI	0.17	0.62	0.17	0.65	2.79	31.30	1.77	7.01
MALME-3M	0.22	1.32	0.22	2.67	1.49	6.89	2.58	60.10
M14	0.42	6.76	0.39	6.01	1.82	9.43	4.55	45.90
MDA-MB-435	0.21	1.71	0.32	6.14	2.94	39.20	2.71	42.80
SK-MEL-2	--	--	--	--	--	--	--	--
SK-MEL-28	0.20	0.62	0.20	0.66	2.62	30.00	9.87	47.50
SK-MEL-5	0.28	3.53	0.28	3.82	2.19	18.90	3.16	36.20
UACC-257	0.24	1.34	0.21	0.90	0.78	21.00	5.74	64.70
UACC-62	0.22	0.83	3.66	4.33	2.65	34.60	3.32	42.30
Ovarian Cancer								
IGROV1	0.21	0.79	0.21	0.74	1.77	18.20	--	--
OVCAR-3	0.20	0.59	0.19	0.61	1.77	5.86	1.93	22.30
OVCAR-4	0.39	4.52	0.41	>100	1.61	5.67	2.66	36.20
OVCAR-5	0.20	0.72	0.40	0.95	3.83	39.20	3.25	37.30
OVCAR-8	0.24	2.80	0.25	4.51	3.01	28.50	11.60	>100
NCI/ADR-Res	0.21	2.80	0.24	0.90	6.12	44.20	2.72	>100
SK-OV-3	1.69	5.64	1.74	5.72	14.80	52.90	10.50	49.00
Renal Cancer								
786-0	0.45	6.08	0.75	6.66	3.38	33.90	1.86	8.33
A498	1.80	6.35	1.80	6.64	--	--	2.15	47.50
ACHN	0.55	4.32	1.52	5.43	2.14	13.50	3.63	42.10
CAKI-1	0.19	0.74	0.20	0.75	1.87	8.00	1.47	6.56
RXF 393	--	--	--	--	1.64	5.69	2.29	25.20
SN12C	0.26	1.75	0.37	6.16	2.44	31.00	2.26	29.00
TK-10	1.82	6.34	1.56	5.98	2.54	26.90	7.29	67.50
UO-31	0.21	0.95	0.23	2.22	2.41	32.50	2.43	48.90
Prostate Cancer								
PC-3	0.18	0.62	0.20	0.663	9.12	55.90	6.82	74.90
DU-145	0.20	0.62	0.18	0.59	5.31	42.70	4.74	45.40
Breast Cancer								
MCF-7	0.16	0.82	0.16	0.77	1.43	6.54	2.48	44.10
MDA-MB-231/ATCC	0.18	0.62	0.18	0.70	2.40	26.10	2.32	39.20
HS 578T	0.26	>100	0.28	>100	--	--	3.80	>100
BT549	0.21	0.74	0.50	4.21	--	--	2.98	38.80
T-47D	0.23	0.74	0.23	0.48	2.37	78.50	2.89	56.80
MDA-MB-468	0.21	0.74	0.21	>100	1.56	6.91	2.37	44.80

2.3. Pharmacology

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 NCI-60 DTP human tumor cell line screen [16,17]. The tumor cells were cultured in RPMI1640 medium supplemented with 5% fetal bovine serum and 2 mM L-glutamine. The tumor cells were inoculated in to 96-well microtiter plates, 100 μ L at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of the individual cell lines [16-19]. After this cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 24 h. Two plates of each cell line are fixed in situ with TCA to represent a measure of the cell population (T₀) before adding the target compounds. The target compounds are dissolved in DMSO and diluted in the test medium to obtain the desired concentration. 100 μ L of each of the test compound solution is now added to the above appropriate cell line microtiter wells and incubated for 48 h at 37 °C, 5% CO₂, 95% air and 100% relative humidity. 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 [17,20-22]. At the present time at NCI the target compounds were tested initially at a single dose at 10 μ M concentration and those promising target compounds are further tested at five dose testing concentrations 0.01, 0.1, 1.0, 10, 100 μ M.

Concentration parameters GI₅₀, TGI and LC₅₀: The NCI re-named the IC₅₀ as GI₅₀. GI₅₀ value represents the concentration of the target compound that causes 50% growth inhibition, that is derived from the formula $100 \times (T - T_0) / (C - T_0) = 50$, where T is the optical density of the target compound after 48 h exposure. T₀ is the optical density at time 0 and C is the control optical density. TGI represents the concentration of the target compound where $100 \times (T - T_0) / (C - T_0) = 0$ and it is the cytostatic effect. LC₅₀ is the concentration of the target compound where $100 \times (T - T_0) / T_0 = -50$. LC₅₀ also signifies the cytostatic effect and the control optical density is not used in the calculation.

3. Experimental

3.1. General

Unless otherwise stated, all chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. Melting points were determined on an Electrothermal MEL-TEMP apparatus and are uncorrected. ¹H-NMR and ¹³C-NMR spectra were recorded in DMSO-d₆ on a Bruker 500 MHz instrument and the chemical shift (δ) values are reported in parts per million (ppm) relative to TMS. A Thermo Scientific LTQ Linear Trap LC/MS/MS system was used for mass spectrometry. UV spectra were recorded on a Beckman-Coulter DU-800 spectrophotometer. Analytical TLC was carried out on Sigma-Aldrich (cat # Z122785-25EA), 0.2 mm percolated silica gel polyester sheets with UV indicator. Elemental analysis was carried out by M-H-W Laboratories, Phoenix, AZ. Analysis of C, H, N were within $\pm 0.4\%$ of theoretical values. The carbon numbering was shown for representative monomer **20a** and for one representative dimer **20b**. All others were referred similarly on the ¹³C-NMR assignments.

3.2. General Procedure-A for *N*₉-[(*Z*)-4'-chloro-2'-butenyl-1'-yl]-6-methoxypurine (**6**)

Synthesis of 6-methoxypurine: To a stirred suspension of 6-chloropurine (5.0 g, 32 mmol) in anhydrous methanol (240 mL) was slowly added 30 W% sodium methoxide in methanol (17.3 g, 320 mmol) at room temperature and then the reaction mixture was refluxed for 18 h. It was cooled to room temperature, neutralized with glacial acetic acid to pH 7.5–8.0 and then evaporated in a rotary evaporator to remove the solvent. The residue was treated with cold water (5 °C) (100 mL), the resulting solid was filtered, thoroughly washed with DI water. The product was crystallized from methanol as brownish white solid (3.5 g), 72% yield. ¹H-NMR: δ 13.39 (1H, br s, NH), 8.50 (1H, s, H-8), 4.10 (3H, s, OCH₃).

To a suspension of 6-methoxypurine (1.51 g, 10 mmol), anhydrous potassium carbonate (2.1 g, 15 mmol) in DMF (50 mL) at room temperature, was added *cis*-1,4-dichloro-2-butene (1.25 g, 10 mmol) and the contents stirred at room temperature for 5 h. The reaction mixture was filtered to remove the potassium carbonate that was also washed with DMF (25 mL). The filtrate and washings combined and evaporated under vacuum. The residue was chromatographed on a column of silica gel and the product was eluted with ethyl acetate: hexane 1:1 v/v. Evaporation of the homogeneous fractions resulted in a residue that was further crystallized from ethyl acetate-hexane as cream white rosettes, (1.3 g), 54% yield, m.p. 104–106 °C. ¹H-NMR: δ 8.54 (1H, s, H-2), 8.36 (1H, s, H-8), 5.89–5.84 (2H, m, HC=CH), 5.4 (2H, s, N-CH₂), 4.10 (3H, s, OCH₃), 2.71 (2H, m, CH₂Cl), 1.10 (3H, t, *J* = 7.5 Hz, OCH₃). ¹³C-NMR: δ 160.22 (C-6), 151.76 (C-4), 151.49 (C-2), 129.66 (C-3'), 128.11 (C-2'), 120.51 (C-5), 53.84 (OCH₃), 40.01 (C-1'), 39.85 (C-4'). Anal. Calcd. for C₁₀H₁₁N₄OCl: C 50.32, H 4.65, N 23.47; Found: C 50.20, H 4.74, N 23.55.

*N*₉-[(*Z*)-4'-Chloro-2'-butenyl-1'-yl]-2,6-dichloropurine (**5a**). A suspension of 2,6-dichloropurine (2.0 g, 11 mmol), anhydrous potassium carbonate (4.6 g, 34 mmol), 1,4-dichloro-2-butyne (2.1 g, 17 mmol) in anhydrous DMF was stirred at r.t. for 6 h. The reaction has been worked-up and purified on a column of silica gel as described in the general procedure-A, to give a major product **5a** and a minor dimeric product **5b**. Compound **5a** was isolated as a cream white solid (1.1g), 46% yield, m.p. 65–67 °C. ¹H-NMR: δ 8.67 (1H, s, H-8), 5.93–5.91 (2H, m, HC=CH), 5.10–5.09 (2H, m, N-CH₂), 4.54–4.52 (2H, m, CH₂Cl). ¹³C-NMR: δ 153.23 (C-2), 150.95 (C-6), 149.59 (C-4), 148.0 (C-8), 130.44 (C-5), 130.24 (C-3'), 127.16 (C-2'), 40.53 (C-1'), 39.13 (C-4'). LC-MS (*m/z*): 277 [M+1]⁺, 100%. Anal. Calcd. for C₉H₇N₄Cl₃: C 38.95, H 2.54, N 20.19; Found: C 39.84, H 2.45, N 20.25.

*N*₉,*N*_{9''}-bis[(*Z*)-2'-Butenyl-1',4'-diyl]-2,6-dichloropurine (**5b**). Compound **5b** was isolated in the above reaction as a brownish white solid (0.45g), 15% yield, m.p. 226–228 °C. ¹H-NMR: δ 8.65(2H, H-8, H-8''), 5.97 (m, 2H, HC=CH), 4.97 (4H, m, 2 × N-CH₂). ¹³C-NMR: δ 153.33 (C-2, C-2''), 150.93 (C-6, C-6''), 149.60 (C-4, C-4''), 148.17 (C-8, C-8''), 130.52 (C-5, C-5''), 127.69 (C-2', C-3'), 40.95 (C-1', C-4'). LC-MS (*m/z*): 431 [M+1]⁺, 100%. Anal. Calcd. for C₁₄H₈N₈Cl₄: C 39.10, H 1.88, N 26.05; Found: C 39.0, H 2.0, N 25.95.

3.3. *N*₉-[(*Z*)-4'-Chloro-2'-butenyl-1'-yl]-2-chloro-6-methoxypurine (7)

Synthesis of 2-chloro-6-methoxypurine: 2,6-dichloropurine (5.0 g, 27 mmol) was reacted with sodium methoxide (30 wt.% 14.6 g, 270 mmol) in anhydrous methanol (250 mL) under reflux for 16 h. The reaction has been worked-up as described above under general procedure-A. The crude product was crystallized from methanol as snow white solid (4.0g), 82% yield. ¹H-NMR: δ 13.52 (1H, br s, NH), 8.42 (1H, s, H-8), 4.1 (3H, s, OCH₃).

2-Chloro-6-methoxypurine (1.9 g, 10 mmol), anhydrous potassium carbonate (2.1 g, 15 mmol), *cis*-1,4-dichloro-2-butene (1.5 g, 12mmol) were stirred in DMF (50 mL) at room temperature for 4 h. The crude product was isolated as described under the general procedure-A. The product was chromatographed on a column of silica gel, eluent ethyl acetate/hexane 1:1 v/v. Cream white solid (1.8g) 64% yield, m.p. 136–138 °C. ¹H-NMR: δ 8.38 (1H, s, H-8), 5.9–5.83 (2H, m, HC=CH), 5.02 (2H, d, *J* = 5.5 Hz, N-CH₂), 4.5 (2H, d, *J* = 5.5 Hz, CH₂Cl), 4.1 (3H, s, OCH₃). ¹³C-NMR: δ 160.73 (C-6), 152.94 (C-2), 151.32 (C-4), 144.04 (C-8), 129.96 (C-3'), 127.64 (C-2'), 119.77 (C-5), 54.87 (OCH₃), 40.05 (C-1'), 39.15(C-4'). Anal. Calcd. for C₁₀H₁₀N₄OCl₂: C 43.93, H 3.69, N 20.51; Found: C 43.85, H 3.75, N 20.45.

*N*₉-[(*E*)-4'-Chloro-2'-butenyl-1'-yl]-2,6-dichloropurine (**8a**). A suspension of 2,6-dichloropurine (2.0 g, 11 mmol), anhydrous potassium carbonate (4.6 g, 34 mmol), *trans*-1,4-dichloro-2-butene (2.15 g, 17 mmol) in anhydrous DMF was stirred at r.t. for 10 h. The reaction was worked-up as described in the general procedure-A. Chromatography of the resulting crude product on a column of silica gel yielded one major product **8a** and a minor dimeric product **8b**. Compound **8a** was isolated as a cream white solid (1.2 g), 40% yield, m.p. 70–72 °C. ¹H-NMR: δ 8.78 (1H, H-8), 6.42 (1H, m, HC=CH), 5.80 (1H, m, HC=CH), 5.0 (2H, d, *J* = 5.5 Hz, N-CH₂), 4.21 (2H, m, CH₂Cl). LC-MS (*m/z*): 277 [M+1]⁺, 100%. Anal. Calcd. for C₉H₇N₄Cl₃: C 38.95, H 2.54, N 20.19; Found C 38.82, H 2.65, N 20.25.

*N*₉,*N*_{9'}-bis[(*E*)-2'-Butenyl-1',4'-diyl]-2,6-dichloropurine (**8b**). Pale brown solid (0.6g), 13% yield, m.p. 240 °C, decomposes. ¹H-NMR: δ 8.77 (2H, s, H-8), 5.97 (2H, m, HC=CH), 4.96 (4H, 2 × N-CH₂). LC-MS (*m/z*): 431 [M+1]⁺, 100%. Anal. Calcd. for C₁₄H₈N₈Cl₄: C 39.10, H 1.88, N 26.05; Found: C 39.20, H 2.0, N 26.15.

*N*₉-[4'-Chloro-2'-butynyl-1'-yl]-6-chloropurine (**9a**). A suspension of 6-chloropurine (2.5 g, 16 mmol), anhydrous potassium carbonate (3.5 g, 25 mmol) and 1,4-dichlorobutylene (3.0 g, 24 mmol) in DMF (150 mL) was stirred at room temperature for 3.0 h. The crude product was isolated as described in the general procedure-A. It was chromatographed on a column of silica gel using ethyl acetate/hexane 1:1 (v/v) as eluents to furnish **9a** as a brownish white solid, 2.3 g, 59% yield, m.p. 92–94 °C. ¹H-NMR: δ 8.83 (1H, s, H-2), 8.77 (1H, s, H-8), 5.32 (2H, t, *J* = 2.0 Hz, N-CH₂), 4.49 (2H, t, *J* = 2.0 Hz, CH₂Cl). ¹³C-NMR: δ 151.79 (C-2), 151.35 (C-6), 149.23 (C-4), 146.74 (C-8), 130.72 (C-5), 80.76 (C-3'), 79.71 (C-2'), 33.43 (C-1'), 30.47 (C-4'). Anal. Calcd. for C₉H₆N₄Cl₂: C 44.84, H 2.51, N 23.24; Found: C 44.75, H 2.64, N 23.15. From the above column, the ethyl acetate/hexane 2:1 v/v and 100% ethyl

acetate eluents furnished the minor dimeric product *N*₉,*N*_{9''}-bis[2'-butynyl-1',4'-diyl]-6-chloropurine (**9b**) as a pale brown solid. 0.7 g, 12% yield, m.p. 220 °C decomposes. ¹H-NMR: δ 8.95 (2H, s, H-2, H-2', H-2''), 8.75 (2H, s, H-8, H-8''), 5.24 (4H, 2 × N-CH₂). ¹³C-NMR: δ 151.9 (C-2, C-2''), 151.5 (C-6, C-6''), 149.4 (C-4, C-4''), 146.9 (C-8, C-8''), 130.8 (C-5, C-5''), 80.5 (C-2', C-3'), 40.9 (C-1', C-4'). Anal. Calcd. for C₁₄H₈N₈Cl₂: C 46.82, H 2.25, N 31.20; Found: C 46.92, H 2.14, N 31.25.

*N*₉-[4'-Chloro-2'-butynyl-1'-yl]-2,6-dichloropurine (**10a**). A suspension of 2,6-dichloropurine (1.9 g, 10 mmol), anhydrous K₂CO₃ (4.2 g, 30 mmol) and 1,4-dichloro-2-butyne (1.85 g, 15 mmol) was stirred in DMF at r.t. for 8 h under argon. The reaction has been worked-up as described in the general procedure-A. The resulting product was chromatographed on a column of silica gel to furnish **10a** as the major product and **10b** as a minor dimeric product. Compound **10a** cream white solid (1.4 g) 50% yield, m.p. 80–82 °C. ¹H-NMR: δ 8.79 (1H, s, H-8), 5.29 (2H, t, *J* = 2.0 Hz, N-CH₂), 4.50 (2H, t, *J* = 2.0 Hz, CH₂Cl). ¹³C-NMR: δ 152.87 (C-2), 151.23 (C-6), 149.91 (C-4), 147.66 (C-8), 130.41 (C-5), 81.11 (C-3'), 79.32 (C-2'), 33.66 (C-1'), 30.46 (C-4'). LC-MS (*m/z*): 275 [M+1]⁺, 100%. Anal. Calcd. for C₉H₅N₄Cl₃: C 39.23, H 1.83, N 20.33; Found: C 39.10, H 1.70, N 20.15.

*N*₉, *N*_{9''}-bis[2'-Butynyl-1',4'-diyl]-2,6-dichloropurine (**10b**). It was isolated as a brownish white solid (0.6g), 14% yield, m.p. 210 °C decomposes. ¹H-NMR: δ 8.78 (2H, s, H-8, H-8''), 5.25 (4H, s, 2 × N-CH₂). LC-MS (*m/z*): 429 [M+1]⁺, 100%. Anal. Calcd. for C₁₄H₆N₈Cl₄: C 39.28, H 1.41, N 26.18; Found: C 39.12, H 1.55, N 26.25.

*N*₉-[4'-Chloro-2'-butynyl-1'-yl]-6-methoxypurine (**11**). 6-Methoxypurine (1.0 g, 7.0 mmol), anhydrous potassium carbonate (1.6 g, 12.0 mmol), 1,4-dichlorobutyne (1.1 g, 9.0 mmol) was stirred at r.t. for 4 h. The crude product was isolated as described in the general procedure-A. Further purification on a column of silica gel using ethyl acetate/hexane 1:1 v/v as the eluent furnished a brownish white solid (1.1g), 70% yield, m.p. 141–143 °C. ¹H-NMR: δ 8.57 (1H, s, H-2), 8.45 (1H, s, H-8), 5.25 (2H, t, *J* = 2.0 Hz, N-CH₂), 4.48 (2H, t, *J* = 2.0 Hz, CH₂Cl), 4.10 (3H, s, OCH₃). ¹³C-NMR: δ 160.31 (C-6), 151.79 (C-2), 151.50 (C-4), 143.07 (C-8), 120.45 (C-5), 80.30 (C-2', C-3'), 53.95 (OCH₃), 32.92 (C-1'), 30.52 (C-4'). Anal. Calcd. for C₁₀H₉N₄OCl: C 50.75, H 3.83, N 23.67; Found: C 50.60, H 3.90, N 23.55.

*N*₉-[4'-chloro-2'-butynyl-1'-yl]-2-chloro-6-methoxypurine (**12**). Brownish white solid, 61% yield, m.p. 143–145 °C. ¹H-NMR: δ 8.48 (1H, s, H-8), 5.23 (2H, t, *J* = 2.0 Hz, N-CH₂), 4.45 (2H, t, *J* = 2.0 Hz, CH₂Cl), 4.11 (3H, s, OCH₃). ¹³C-NMR: δ 160.85 (C-6), 152.64 (C-2), 151.61 (C-4), 143.82 (C-8), 119.75 (C-5), 80.66 (C-3'), 79.88 (C-2'), 54.98 (OCH₃), 33.18 (C-1'), 30.50 (C-4'). Anal. Calcd. for C₁₀H₈N₄OCl₂: C 44.30, H 2.97, N 20.67; Found: C 44.15, H 3.15, N 20.55.

3.4. General procedure B for *N*₉-[(*E*)-2',3'-dibromo)-4'-chloro-2'-butenyl-1'-yl]-6-chloropurine (**13**)

A suspension of **9a** (0.242 g, 1.0 mmol), pyridiniumtribromide (0.4 g, 1.3 mmol) in anhydrous dichloromethane (100 mL) was cooled to −10 °C while stirring. Anhydrous methanol (50 mL) was added drop wise during 15–20 minutes. The reaction mixture was allowed to warm-up and stirred for

20 h in a fume hood. The clarified reaction mixture was evaporated on a rotary evaporator at 30–35 °C without any quenching with sodium thiosulfate. The product was purified on a column of silica gel using ethyl acetate - light petroleum ether 1:1, 2:1 v/v as the eluents. It was further crystallized from ethyl acetate-light petroleum ether as a white crystalline solid 0.22 g, 54% yield, m.p. 88–90 °C. ¹H-NMR: δ 8.80 (1H, s, H-2), 8.70 (1H, s, H-8), 5.40 (2H, s, N-CH₂), 4.71 (2H, s, CH₂Cl). ¹³C-NMR: δ 151.70 (C-2), 151.30 (C-6), 149.0 (C-4), 146.7 (C-8), 130.70 (C-5), 121.30 (C-3'), 119.40 (C-2'), 50.40 (C-1'), 49.70 (C-4'). Anal. Calcd. for C₉H₆N₄Br₂Cl₂: C 26.96, H 1.51, N 13.98; Found: C 26.80, H 1.65, N 14.15. The pyridinium salts were retained on the column.

*N*₉-[(*E*)-2',3'-Dibromo-4'-chloro-2'-butenyl-1'-yl]-6-methoxypurine (**14**). Compound **11** was used as the starting material and the above bromination procedure was followed. A crystalline white solid resulted, 65% yield, m.p. 127–129 °C. ¹H-NMR: δ 8.56 (1H, s, H-2), 8.41 (1H, s, H-8), 5.45 (2H, s, N-CH₂), 4.72 (2H, s, CH₂Cl), 4.11 (3H, s, OCH₃). ¹³C-NMR: δ 160.29 (C-6), 152.01 (C-4), 151.94 (C-2), 143.93 (C-8), 121.59 (C-5), 121.33 (C-3'), 119.46 (C-2'), 53.97 (OCH₃), 50.42 (C-1'), 49.75 (C-4'). LC-MS (*m/z*): 397[M+1]⁺, 100%. Anal. Calcd. for C₁₀H₉N₄OBr₂Cl: C 30.29, H 2.29, N 14.13; Found: C 30.10, H 2.34, N 14.05.

*Synthesis of N*₉-(tetrahydropyran-2-yl)-6-chloropurine (**25**). A suspension of 6-chloropurine (5.0 g, 32 mmol), 3,4-dihydro-2H-pyran (5.4 g, 64 mmol) and pyridinium-*p*-toluenesulfonate (PPTS) (3.3 g, 13 mmol) in anhydrous dichloromethane (250 mL) was stirred at room temperature in an atmosphere of argon for 64 h. The original suspension transformed in to a clear liquid and the starting material disappeared. The reaction mixture was evaporated and the residue was chromatographed on a column of silica gel. The THP ether was eluted with 100% ethyl acetate and that was isolated as a semi-solid. It solidified as a pale yellow soft solid upon cooling in a freezer (7.0 g), 91% yield. Homogeneous on TLC on silica gel plate, R_f 0.58, mobile phase 100% ethyl acetate. The starting material 6-chloropurine under the same conditions was slow moving, R_f 0.12. ¹H-NMR: δ 8.92 (1H, s, H-2), 8.81 (1H, H-8), THP protons: 5.81–5.77 (1H, m), 4.01–3.99 (1H, m), 3.77–3.68 (1H, m), 2.37–2.28 (1H, m), 2.04–1.96 (2H, m), 2.04 (1H, m), 2.03 (2H, m).

3.5. General Procedure C for Synthesis of *N*₉-(tetrahydropyran-2-yl)-6-(4-methoxyphenyl)purine (**26**)

Suzuki-Miyaura cross coupling reaction: A stirred suspension of 6-chloro-9-(tetrahydropyran-2-yl) purine **25**, (3.5 g, 15 mmol), 4-methoxyphenyl boronic acid (3.35 g, 22 mmol), anhydrous potassium carbonate (3.1 g, 22 mmol), tetrakis(triphenylphosphine)palladium (0), Pd (PPh₃)₄, (0.85 g, 0.74 mmol) in anhydrous toluene (150 mL) was gradually heated to 100 °C during 1 hr and then maintained at that temperature for 18 h. The TLC, silica gel plate, indicated the disappearance of the starting material R_f 0.45 and the formation of the new product R_f 0.54, mobile phase ethyl acetate-hexane 7:3 v/v. The reaction mixture was filtered while warm to remove the potassium and boron salts. The filtrate was concentrated and the resulting residue was crystallized from ethyl acetate-methanol 1:1 v/v as a brownish white solid (4.0g), 88% yield, m.p. 146–148 °C. ¹H-NMR: δ 8.93 (1H, s, H-2), 8.87–8.82 (2H, m, Ar-H), 8.70 (1H, s, H-8), 7.18–7.13 (2H, m, Ar-H), 4.06–4.03 (1H, m, THP), 3.87

(3H,s,OCH₃), 3.76–3.71(1H, m, THP), 2.37–2.31 (1H, m, THP), 2.04–1.99(2H, m, THP), 1.81–1.73 (1H, m, THP), 1.67–1.79 (2H, m,THP).

Synthesis of 6-(4-Methoxyphenyl)purine (28). To a suspension of compound **26** (3.2 g, 10 mmol) in methanol (100 mL) was added acetyl chloride (0.2 mL, 2.8 mmol) and the contents stirred overnight at room temperature for 24 h. The reaction mixture was evaporated, the residue was treated with water (100 mL) and the pH was adjusted to 7.5–8.0 with a saturated solution of sodium bicarbonate in water. The resulting solid was filtered, washed with DI water (100 mL) followed by cold ethanol (25 mL), hexane (25 mL) and dried under vacuum, brownish white solid (1.9 g), 81% yield. No further purification was necessary as the product was found to be homogeneous on silica gel TLC, R_f 0.3, mobile phase ethyl acetate-hexane 7:3 v/v. ¹H-NMR: δ 8.97 (1H, s, H-2), 8.82–8.79 (2H, m, Ar-H), 8.75 (1H, s, H-8), 8.0 (1H, broad, NH), 7.2–7.17 (3H, m, Ar-H), 3.88 (3H, s, OCH₃).

N₉-[(Z)-4'-Chloro-2'-butenyl-1'-yl]-6-(4-methoxyphenyl)purine (15a). A suspension of 6-(4-methoxyphenyl)purine (0.68 g, 3.0 mmol), anhydrous potassium carbonate (1.0 g, 7.2 mmol), *cis*-1,4-dichloro-2-butene (0.42 g, 3.4 mmol) was stirred at room temperature under argon atmosphere for 4h. The reaction mixture was worked-up as described in the general procedure-A. The resulting residue was chromatographed on a column of silica gel. Fractions 3–4 (100 mL) each from ethyl acetate-hexane (1:1) v/v furnished a brownish white solid **15a** (0.41 g), 43% yield, m.p. 129–131 °C. ¹H-NMR: δ 8.92 (1H, s, H-2), 8.87–8.82 (2H, m, Ar-H), 8.61 (1H, s, H-8), 7.16–7.13 (2H, s, Ar-H), 5.92–5.89 (2H, m, HC=CH), 5.09–5.07 (2H, d, *J* = 9.0 Hz, N-CH₂), 4.54–4.52 (2H, m, CH₂Cl), 3.86(3H, s, OCH₃). ¹³C-NMR: δ 161.61 (C-13), 152.42 (C-4), 151.86 (C-6), 145.44 (C-8), 131.07 (C-11, C-15), 129.74 (C-3'), 129.62 (C-10), 128.08 (C-2'), 127.82 (C-5), 114.05 (C-12, C-14), 55.32 (OCH₃), 39.68 (C-1'), 39.22(C-4'). LC-MS (*m/z*): 315 [M+1]⁺, 100%. Anal. Calcd. for C₁₆H₁₅N₄OCl: C 61.05, H 4.80, N 17.80; Found: C 61.10, H 4.92, N 17.90.

N₉,N_{9''}-bis[(Z)-2'-Butenyl-1'4'-diyl]-6-(4-methoxyphenyl)purine (15b). The fractions 5–8 from the above column chromatography yielded a brownish white solid (0.1 g), 11% yield, m.p. 163–165 °C. ¹H-NMR: δ 8.93 (2H, s, H-2, H-2''), 8.89–8.84 (4H, m, Ar-H), 8.72 (2H, s, H-8, H-8''), 7.17–7.14 (4H, m, Ar-H), 5.99–5.96 (2H, m, HC=CH), 5.29–5.27 (4H, d, *J* = 10.0 Hz, 2 × N-CH₂), 3.87 (6H, s, C-13-OCH₃, C-13''-OCH₃). ¹³C-NMR: δ 161.63 (C-13, C-13''), 152.45 (C-4, C-4''), 151.98 (C-6, C-6''), 151.73 (C-2, C-2''), 145.66 (C-8, C-8''), 131.10 (C-11, C-11'', C-15, C-15''), 129.70 (C-10, C-10''), 128.03 (C-2', C-3'), 127.85 (C-5, C-5''), 114.09 (C-12, C-12'', C-14, C-14''), 55.34 (C-13-OCH₃, C-13''-OCH₃), 40.20 (C-1', C-4'). LC-MS (*m/z*): 505 [M+1]⁺, 100%. Anal. Calcd. for C₂₈H₂₄N₈O₂: C 66.65, H 4.80, N 22.21; Found: C 66.52, H 4.92, N 22.33.

3.6. Synthesis of N₉-(tetrahydropyran-2-yl)-6-(4-fluorophenyl)purine (27)

Suzuki-Miyaura cross coupling reaction: 9-(Tetrahydropyran-2-yl)-6-chloropurine (4.8 g, 20 mmol), 4-fluorophenyl boronic acid (4.2 g, 30 mmol), Pd(PPh₃)₄, (1.0 g, 0.9 mmol) in anhydrous dimethoxy-ethane (200 mL) was added a 2.7 molar saturated Na₂CO₃ solution in water (11.2 mL, 30 mmol). The reaction mixture was gradually heated to reflux during 2 h in an oil bath and then

maintained at reflux for 7 h. Reaction was worked-up and purified as above to yield a brownish white solid (5.0 g), 83% yield. m.p. 146–148 °C. ¹H-NMR: δ 9.0 (1H, s, H-2), 8.93–8.89 (2H, m, Ar-H), 8.88 (1H, s, H-8), 7.46–7.42 (2H, m, Ar-H), THP protons: 5.85–5.82 (1H, m), 4.06–4.03 (1H, m), 3.77–3.72 (1H, m), 2.38–2.35 (1H, m), 2.06–1.99 (2H, m), 1.84–1.72 (1H, m), 1.63–1.61 (2H, m).

6-(4-Fluorophenyl)purine (29). To a stirred suspension of 9-(tetrahydropyran-2-yl)-6-(4-fluorophenyl) purine (**27**, 3.75 g, 13 mmol) in methanol (200 mL) at room temperature was added acetyl chloride (0.4 mL, 5.6 mmol) and the reaction was worked-up as described in **26**. A brownish white solid resulted (2.6 g), 96% yield. The product was found homogeneous on TLC on a silica gel plate, R_f 0.3, mobile phase ethyl acetate-hexane (7:3) v/v. ¹H-NMR: δ 8.94 (3H, s, H-2 and 2H Ar-H), 8.92 (1H, br, NH), 8.65 (1H, s, H-8), 7.45–7.40 (2H, m, Ar-H).

N₉-(Z)-4'-Chloro-2'-butenyl-1'-yl]-6-(4-fluorophenyl)purine (16a). A suspension of 6-(4-fluorophenyl) purine **29** (0.65 g, 3.0 mmol), anhydrous potassium carbonate (1.0 g, 7.2 mmol), *cis*-1,4-dichloro-2-butene (0.413 g, 3.3 mmol) in DMF (50 mL) was stirred under argon at room temperature for 3.5 h. The reaction was worked-up as described in general procedure A. The crude reaction mixture contained a major product **16a** and a minor dimeric product **16b**. The above crude product upon crystallization from ethyl acetate-methanol furnished the dimeric product **16b** as feathery brownish white needles. The mother liquor was concentrated and chromatographed on a column of silica gel using ethyl acetate-light petroleum ether (1:1, 2:1 v/v) as the eluents that furnished a brownish white solid of **16a** (0.4 g), 43% yield, m.p. 94–96 °C. ¹H-NMR: δ 8.99 (1H, s, H-2), 8.93–8.90 (2H, m, Ar-H), 8.67 (1H, s, H-8), 7.45–7.41 (2H, m, Ar-H), 5.93–5.91 (2H, m, HC=CH), 5.10–5.09 (2H, d, *J* = 5.5 Hz, N-CH₂), 4.54–4.52 (2H, m, CH₂Cl). ¹³C-NMR: δ 164.80 (d, ¹*J*_{CF} = 249 Hz, C-13), 152.13 (C-4), 151.75 (C-2), 151.39 (C-6), 146.09 (C-8), 131.85 (d, ⁴*J*_{CF} = 3.8 Hz, C-10), 131.75 (d, ³*J*_{CF} = 8.8 Hz, C-11, C-15), 129.99 (C-5), 129.82 (C-3'), 127.97 (C-2'), 115.73 (d, ²*J*_{CF} = 21.4 Hz, C-12, C-14), 39.74 (C-1'), 39.21 (C-4'). LC-MS (*m/z*): 303 [M+1]⁺, 10%, 267 [M+1]⁺-HCl, 100%. Anal. Calcd. for C₁₅H₁₂N₄FCl: C 59.51, H 4.0, N 18.51; Found: C 59.40, H 4.10, N 18.45.

N₉,N_{9'}-bis[(Z)-2'-Butenyl-1',4'-diyl]-6-(4-fluorophenyl)purine (16b). 0.27 g, 18% yield, m.p. 198–200 °C. ¹H-NMR: δ 8.99 (2H, s, H-2, H-2'), 8.94–8.92 (4H, m, Ar-H), 8.77 (2H, s, H-8, H-8'), 7.46–7.42 (4H, m, Ar-H), 6.01–5.99 (2H, m, HC=CH), 5.31–3.30 (4H, d, *J* = 5.5 Hz, 2 × N-CH₂). ¹³C-NMR: δ 164.83 (d, ¹*J*_{CF} = 249 Hz, C-13, C-13'), 152.27 (C-4, C-4'), 151.76 (C-2, C-2'), 151.44 (C-6, C-6'), 146.34 (C-8, C-8'), 131.90 (d, ⁴*J*_{CF} = 3.8 Hz, C-10, C-10'), 131.79 (d, ³*J*_{CF} = 18.9 Hz, C-11, C-11', C-15, C-15'), 130.09 (C-5, C-5'), 128.04 (C-2', C-3'), 115.80 (d, ²*J*_{CF} = 21.4 Hz, C-12, C-12', C-14, C-14'), 40.29 (C-1', C-4'). LC-MS (*m/z*): 481 [M+1]⁺, 100%. Anal. Calcd. for C₂₆H₁₈N₈F₂: C 64.99, H 3.78, N 23.32; Found: C 65.10, H 3.82, N 23.45.

N₉-(E)-4'-Chloro-2'-butenyl-1'-yl]-6-(4-methoxyphenyl)purine (17). Cream white needles, 63% yield, m.p. 112–114 °C. ¹H-NMR: δ 8.92 (1H, s, H-2), 8.87–8.82 (2H, m, Ar-H), 8.61 (1H, s, H-8), 7.16–7.13 (2H, s, Ar-H), 6.19–6.09 (1H, m, HC=CH), 5.83–5.73 (1H, m, HC=CH), 4.99–4.98 (2H, d, *J* = 9.0 Hz, N-CH₂), 4.23–4.20 (2H, m, CH₂Cl), 3.86 (3H, s, OCH₃). ¹³C-NMR: δ 161.62 (C-13), 152.47 (C-4), 151.91 (C-6),

151.82 (C-2), 145.66 (C-8), 131.09 (C-11, C-15), 129.59 (C-10), 129.50 (C-3'), 129.02 (C-2'), 127.83 (C-5), 114.06 (C-12,14), 55.33 (C-13-OCH₃), 44.08 (C-1'), 43.78 (C-4'). LC-MS (*m/z*): 277 [M+1]⁺, 100%. Anal. Calcd. for C₁₆H₁₅N₄OCl: C 61.05, H 4.80, N 17.80; Found: C 61.15, H 4.95, N 17.95.

*N*₉-[(*E*)-4'-Chloro-2'-butenyl-1'-yl]-6-(4-fluorophenyl)purine (**18a**). A suspension of 6-(4-fluorophenyl) purine (0.5 g, 2.3 mmol), anhydrous potassium carbonate (0.65 g, 4.7 mmol), *trans*-1,4-dichloro-2-butene (0.33 g, 2.6 mmol) in DMF (40 mL) was stirred at room temperature under argon for 22 h. The reaction has been worked-up as described in the general procedure. The resulting product was chromatographed on a column of silica gel using ethyl acetate - light petroleum ether (1:1) v/v as an eluent. Fractions of 100 mL were collected. The fractions 3-4 yielded **18a** as a cream white solid, homogeneous on silicagel TLC, mobile phase ethyl acetate-hexane 7:3 v/v, R_f 0.69 (0.4g), 57% yield, m.p. 73–75 °C. ¹H-NMR: δ 8.98 (1H, s, H-2), 8.94–8.91 (2H, m, Ar-H), 8.68 (1H, s, C-8), 7.45–7.42 (2H, m, Ar-H), 6.16–6.12 (1H, m, HC=CH), 5.82–5.79 (1H, m, HC=CH), 5.01–5.0 (2H, d, *J* = 5.0 Hz, N-CH₂), 4.22–4.20 (2H, m, CH₂Cl). ¹³C-NMR: δ 164.80 (d, ¹*J*_{CF} = 250 Hz, C-13), 152.16 (C-4), 151.80 (C-2), 151.43 (C-6), 146.27 (C-8), 131.85 (d, ⁴*J*_{CF} = 2.52 Hz, C-10), 131.75 (d, ³*J*_{CF} = 8.8 Hz, C-11, C-15), 129.96 (C-5), 129.59 (C-3'), 128.87 (C-2'), 115.71 (d, ²*J*_{CF} = 21.4 Hz, C-12, C-14), 44.04 (C-1'), 43.85 (C-4'). LC-MS (*m/z*): 303 [M+1]⁺, 100%. Anal. Calcd. for C₁₅H₁₂N₄FCl: C 59.51, H 4.0, N 18.51; Found: C 59.25, H 4.16, N 18.65.

*N*₉,*N*_{9'}-bis[(*E*)-2'-Butenyl-1',4'-diyl]-6-(4-fluorophenyl)purine (**18b**). From the above chromatography, fractions 6–10 yielded this dimer **18b**, R_f 0.1, brownish white solid, (0.15 g), 13% yield, m.p. 217–219 °C. ¹H-NMR: δ 8.95 (2H, s, H-2, H-2''), 8.93–8.90 (4H, m, Ar-H), 8.65 (2H, s, H-8, H-8''), 7.46–7.42 (4H, m, Ar-H), 5.98 (2H, m, HC=CH), 4.97–4.96 (4H, m, 2 × N-CH₂). ¹³C-NMR: δ 164.86 (d, ¹*J*_{CF} = 252 Hz, C-13, C-13''), 152.61 (C-4, C-4''), 151.75 (C-6, C-6''), 151.61 (C-2, C-2''), 144.04 (C-8, C-8''), 131.44 (d, ³*J*_{CF} = 8.8 Hz, C-11, C-11'', C-15, C-15''), 131.32 (d, ⁴*J*_{CF} = 2.52 Hz, C-10, C-10''), 130.06 (C-5, C-5''), 127.90 (C-2', C-3'), 115.06 (d, ²*J*_{CF} = 21.4 Hz, C-12, C-12'', C-14, C-14''), 43.88 (C-1', C-4'). LC-MS (*m/z*): 481 [M+1]⁺, 100%. Anal. Calcd. for C₂₆H₁₈N₈F₂: C 64.99, H 3.78, N 23.32; Found: C 64.82, H 3.85, N 23.45.

*N*₉-[4'-Chloro-2'-butynyl-1'-yl]-6-(4-methoxyphenyl)purine (**19**). White solid, 80% yield, m.p. 119–121 °C. ¹H-NMR: δ 8.93 (1H, s, H-2), 8.87–8.82 (2H, m, Ar-H), 8.70 (1H, s, H-8), 7.18–7.13 (2H, m, Ar-H), 5.31 (2H, t, *J* = 3.5 Hz, N-CH₂), 4.50 (2H, m, CH₂Cl), 3.87 (3H, s, OCH₃). ¹³C-NMR: δ 161.70 (C-13), 152.67 (C-4), 152.0 (C-2), 151.58 (C-6), 145.19 (C-8), 131.13 (C-11, C-15), 129.49 (C-10), 127.67 (C-5), 114.11 (C-12, C-14), 80.34 (C-3'), 80.28 (C-2'), 55.34 (OCH₃), 32.77 (C-1'), 30.55 (C-4'). LC-MS (*m/z*): 313 [M+1]⁺, 100%. Anal. Calcd. for C₁₆H₁₃N₄OCl: C 61.44, H 4.19, N 17.91; Found: C 61.35, H 4.30, N 17.85.

*N*₉-[4'-Chloro-2'-butynyl-1'-yl]-6-(4-fluorophenyl)purine (**20a**). A brownish white solid, 64% yield, m.p. 128–130 °C. ¹H-NMR: δ 9.02 (1H, s, H-2), 8.92–8.89 (2H, m, Ar-H), 8.75 (1H, s, H-8), 7.46–7.42 (2H, m, Ar-H), 5.33 (2H, t, *J* = 2.0 Hz, N-CH₂), 4.50 (2H, t, *J* = 2.0 Hz, CH₂Cl). ¹³C-NMR: δ 164.86 (d, ¹*J*_{CF} = 249 Hz, C-13), 151.99 (C-2), 151.83 (C-4), 151.65 (C-6), 145.80 (C-8), 131.79 (d, ³*J*_{CF} = 8.8 Hz, C-11, C-15), 131.68 (d, ⁴*J*_{CF} = 2.52 Hz, C-10), 129.87 (C-5), 115.75 (d, ²*J*_{CF} = 21.4 Hz,

C-12, C-14), 80.43 (C-3'), 80.14 (C-2'), 32.86 (C-1'), 30.52 (C-4'). LC-MS (m/z): 301 $[M+1]^+$, 100%. Anal. Calcd. for $C_{15}H_{10}N_4FCl$: C 59.91, H 3.35, N 18.63; Found: C 60.0, H 3.40, N 18.55.

N_9,N_9'' -[2'-butynyl-1',4'-diyl]-6-(4-fluorophenyl)purine (**20b**). A light brown solid, 11% yield, m.p. 225–227 °C. 1H -NMR: δ 8.99 (2H, s, H-2, H-2''), 8.93–8.90 (4H, m, Ar-H), 8.76 (2H, s, H-8, H-8''), 7.47–7.43 (4H, m, Ar-H), 6.01–5.30 (4H, s, $2 \times N-CH_2$). LC-MS (m/z): 479 $[M+1]^+$, 100%. Anal. Calcd. for $C_{26}H_{16}N_8F_2$: C 65.27, H 3.37, N 23.42; Found: C 65.15, H 3.45, N 23.55.

4. Conclusions

On the purine base selection, a strong electron withdrawing 2,6-dichloro system on the purine base is helpful for the cytotoxicity, ex: compounds **5a**, **5b**, **8b**, **10a**, **10b**. This also suggests that 2,6-dichloropurine base has significant potential for further work on the synthesis of anti-cancer compounds. The simultaneous electron withdrawing chlorine at the 2-position and electron donating methoxy group at 6-position on the purine ring did not contribute to the cytotoxic activity of the examples studied, compounds **7**, **12**. A 6-methoxy group on the purine base did not contribute to the cytotoxic activity in compounds **6** or **11**, however, a 6-methoxypurine bearing a chloromethyl vinylic dibromide moiety (compound **14**) exhibited excellent cytotoxic activity. This is the first report of activity in such a molecule and hence has a potential for further exploration in this direction. The 6-(4-methoxyphenyl)purine moiety elicited very potent activity (compound **19**). A riboside of this base also exhibited excellent cytostatic activity [10]. The consistency of this purine base in eliciting tumor inhibiting properties for wide range of cell lines is a very important finding from our work and from that reported in [10]. It is important to note that in our case the linker is an acyclic unsaturated butyne with a chloromethyl group and in the other case [10] it is a natural ribose sugar. Hence this purine base may be considered as potential candidate for future anti-cancer drug development work. The 6-(4-fluorophenyl)purine unit did not elicit any significant cytotoxicity in the compounds studied (**16a**, **20a**, **20b**). This suggests electron withdrawing 4-fluoro group on the phenyl ring was not helpful, a striking difference with the beneficial 4-methoxy group.

In the linker selection, the methylchloromethylbutyne linker emerged as a viable non-sugar unsaturated linker (compounds **10a**, **19**). Even the dimeric compound with the butyne linker, **10b** exhibited cytotoxicity indicating this linker with the triple bond is important. Similarly the methylchloromethyl-*cis*-butyne linker compound **5a**, its dimer **5b** demonstrated excellent cytotoxic activity. The methylchloromethyl-*trans*-butene linker compound **17** and the dimer **8b** elicited good cytotoxic activity, but in other cases **8a** did not contribute any activity. The mechanism of action although not determined yet for our molecules may be different from purine nucleosides with natural sugars. Further useful structural modifications of the active purines **5a**, **5b**, **14** and **19** is in progress.

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Sample Availability: Samples of the compounds described in the experimental are available from the authors.

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