Arch. Pharm. Res. Vol. 17, No. 3, pp. 170-174, 1994

Synthesis and Biological Activity of 6-Substituted-2-Oxo-Purine Nucleosides

Sang Jun Lee[1], Jong Bae Kim[2], Young Ho Cho[2] and Jung Han Kim[1]

[1]*Department of Food and Biotechnology & Bioproducts Research Center, Yonsei University, Seoul 120-749, Korea,* [2]*Animal Resource Research Center, Kun Kuk University, Seoul 133-170, Korea*

(Received February 25, 1994)

We have synthesized various 6-substituted-2-oxo-purine nucleosides from key intermediate, 6-[(4-methylphenylthio)-2-oxo-9-(2,3,5-tri-o-acetyl-β-D-ribofuranosyl)]-2,3-dihydropurine in relatively high yields by one step nucleophilic substitution. Various isoguanosine, xanthosine analogs and other 2-oxo-purine nucleosides containing nitrogen, sulfur and oxygen at C-6 of purine base were easily obtained by this method. The structures of the products were established on the basis of their spectral data studies. And cytotoxicity of resulting synthetic 6-substituted-2-oxo-purine nucleosides against some tumor cell-lines was examined. ED_{50} values of these synthetic compounds were above 100 μg/ml except isoguanosine, N^6-methyl isoguanosine and thioxanthosine analogs.

Key words: 6-Substituted-2-oxo-purine nucleosides, Nucleophilic substitution, Isoguanosine analogs, 6-[(4-methylphenylthio)-2-oxo-9-(2,3,5-tri-o-acetyl-β-D-ribofuranosyl]-2,3-dihydropurine, Nucleophilic substitution

INTRODUCTION

It was reported that 2-oxo-6-amino purine nucleosides, isoguanosine, have various biological activities (Frederik *et al.*, 1981). Specially we newly reported that isoguanosine has considerable antitumor activity (Kim *et al.*, 1994) *in vitro* and *in vivo* and after the finding that 2-oxo-6-thiopurine showed a significant antitumor activity, attention was directed toward the synthesis of 6-thiopurine nucleoside derivatives of naturally occurring bases and nucleosides (Kazunobu *et al.*, 1975). Therefore, it is expected that synthetic analogs of these compounds have various biological activities involving antitumor activity. But the studies on the synthesis of 6-substituted-2-oxo purine nucleosides have not been developed. For the synthesis of 6-substituted-2-oxo-purine nucleosides, the major synthetic step is to introduce oxo group at C-2 of purine base in high yield. Several methods for the introduction of oxo group at C-2 of purine base have been reported in the literature. Some of the methods are based on the relatively inaccessible 5-amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide as starting material (Akihiro *et al.*, 1978) and some involve the modification of other 9-(β-D-ribofuranosyl)purines such as 2-aminoadenosine (Davoll, 1951), adenosine 1-oxide (Kazunobu *et al.*, 1975) and 6-chloro-2-iodo-9-(β-D-ribofuranosyl) purine (Nair *et al.*, 1985). But these synthetic methods have disadvantages such as low yields and have not been designed for the introduction of various substituent groups at C-6 of purine base. Recently K. J. Divakar(Divakar *et al.*, 1991) readly prepared key intermediate, 6-[(4-methylphenylthio)-2-oxo-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl]-2,3-dihydropurine (IV) for synthesis of isoguanosine from guanosine in 4 synthetic steps with 64% overall yield (Scheme). We thought that toluenethiol derivatives IV might serve as a good substrate for the synthesis of various 6-substituted-2-oxo purine nucleosides.

In this paper, we report a facile synthesis of 2-oxo purine nucleosides containing nitrogen, sulfur or oxygen atom at C-6 of purine base by nucleophilic substitution using key intermediate IV (Scheme). And cytotoxic activity of resulting synthetic 6-substituted-2-oxo-purine nucleosides against some tumor cell-lines was examined.

RESULTS AND DISCUSSION

Chemistry

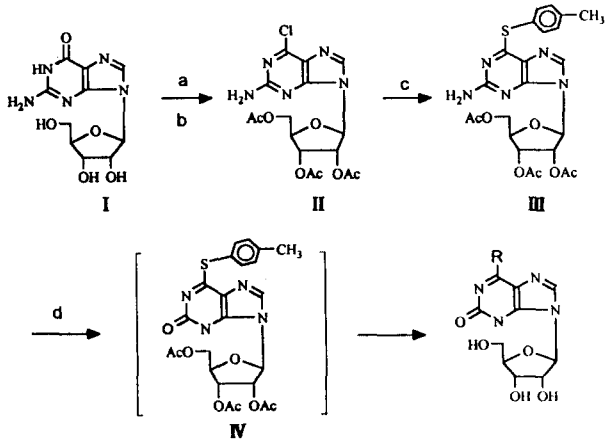
We obtained various sulfur-containing purine nuc-

Correspondence to: Jung Han Kim, Department of Food and Biotechnology, Yonsei University, Seoul 120-749, Korea

Table I. Reaction conditions and properties of 6-substituted-2-oxo-purine nucleosides

No.	6-substituents	reaction time	reaction temp (℃)	yield (%)	molecular formular	mp. (℃)	TLC (Rf)[a]
1	-SH	24	70	42	$C_{10}H_{14}N_4O_5S$	150(decomp.)	0.51
2	$-SCH_3$	20	60	41	$C_{11}H_{14}N_4O_5S$	163(decomp.)	0.58
3	$-SCH_2CH_3$	20	50	62	$C_{12}H_{16}N_4O_5S$	145	0.64
4	$-SCH_2CH_2OH$	12	70	58	$C_{16}H_{16}N_4O_5S$	213	0.45
5	-S◎CH_3	12	r.t.	87	$C_{17}H_{18}N_4O_5S$	201	0.68
6	$-SCH_2$◎	12	70	47	$C_{17}H_{18}N_4O_5S$	187	0.69
7	$-OCH_3$	12	reflux	91	$C_{11}H_{14}N_4O_6$	193-195	0.53
8	$-OCH_2CH_3$	17	reflux	41	$C_{12}H_{16}N_4O_6$	198(decomp.)	0.56
9	-CN	7	60	31	$C_{11}H_{11}N_5O_5$	205(decomp.)	0.43
10	$-NH_2$	20	65	73	$C_{10}H_{13}N_5O_5$	236	0.46
11	$-NHCH_3$	3	r.t.	90	$C_{11}H_{15}N_5O_5$	180	0.49
12	$-NH(CH_3)_2$	3	50	93	$C_{12}H_{17}N_5O_5$	231(decomp.)	0.46
13	$-NHCH(CH_3)_2$	7	70	72	$C_{13}H_{19}N_5O_5$	167-169	0.57
14	$-NHCH_2CH_3$	3	50	89	$C_{12}H_{17}N_5O_5$	212(decomp.)	0.52
15	$-NHCH_2CH_2CH_3$	3	60	79	$C_{13}H_{19}N_5O_5$	170-172	0.55
16	$-NH(CH_2)_3CH_3$	3	60	83	$C_{14}H_{21}N_5O_5$	150-152	0.61
17	$-NH(CH_2)_4CH_3$	5	70	75	$C_{15}H_{23}N_5O_5$	151-153	0.62
18	-NH≈	7	60	79	$C_{16}H_{17}N_5O_5$	156-158	0.64
19	$-NHCH_2CH_2NH_2$	3	40	47	$C_{13}H_{18}N_6O_5$	210(decomp.)	0.26
20	$-NHCH_2CH_2OH$	7	50	72	$C_{13}H_{17}N_5O_6$	198(decomp.)	0.46
21	-NH◎	40	reflux	54	$C_{16}H_{17}N_5O_5$	156-158	0.50
22	$-NHCH_2$◎	15	60	75	$C_{17}H_{19}N_5O_5$	143-145	0.60
23	$-NH_2CH_2$◇	6	75	52	$C_{15}H_{18}N_6O_5$	174-175	0.61

[a]Solvents; BuOH : AcOH : H_2O (4 : 1 : 5)



R: 1. SH, 2. SCH_3, 3. SCH_2CH_3, 4. SCH_2CH_2OH, 5. S◎CH_3, 6. SCH_2◎ 7. OCH_3, 8. OCH_2CH_3, 9. CN, 10. NH_2, 11. $NHCH_3$, 12. $NH(CH_3)_2$, 13. $NHCH(CH_3)_2$, 14. $NHCH_2CH_3$, 15. $NH(CH_2)_2CH_3$, 16. $NH(CH_2)_3CH_3$, 17. $NH(CH_2)_4CH_3$ 18. NH≈, 19. $NHCH_2CH_2NH_2$, 20. $NHCH_2CH_2OH$, 21. NH◎ , 22. $NHCH_2$◎ , 23. $NHCH_2$◇

(a) Ac_2O, Pyridine, DMF, 70℃

(b) $POCl_3$, N,N-dimethylaniline, CH_3CN, 100℃

(c) toluenethiol, TEA, DMF, 100℃

(d) 50% AcOH, $NaNO_2$, 50℃

Scheme 1

leosides from intermediate IV by one step substitution reaction. The first synthesis of thiopurine nucleosides, 6-thioinosine and 6-thioguanosine, described by Fox and his coworker (Fox *et al.*, 1958) was accomplished by the thiation of protected inosine and guanosine with phosphorus pentasulfide and Kazunobu Miura (Kazunobu *et al.*, 1975) has described synthesis of its analogs. But these synthetic methods could not avoid relatively low yield.

As seen in Table I, treatment of IV with ethanol saturated with H_2S afforded light yellow crystal 6-thioxanthosine (1) in 42% yield. In similar manner, various 6-sulfur-containing purine nucleosides were easily obtained in relatively high yields. Treatment of IV with sodium cyanide in DMF gave the 6-cyano derivative in relatively high yield. The presence of nitrile absorptions in the IR spectra of cyano compound is characteristic. Similar result of the nitrile absorption has been observed in a 6-cyano-9-β-D-ribofuranosyl purine (Akira *et al.*, 1980) and also we confirmed that toluenethiol was readly substituted with methoxide and ethoxide with high yields.

For the synthesis of series of N^6-substituted isoguanosine which has considerable antitumor activity, key intermediate IV in the present study was reacted with various amines by one step substitution reaction. As seen in Table II, isoguanosine analogs were prepared more directly by heating the intermediate IV with various amines in ethanol or aqueous ethanol solution in high yield. Especially we knew that toluenethiol substituent was easily substituted with bulky amines such

Table II. Compound data of 6-substituted-2-oxo-purine nucleosides

Comp. No	UV λ_{max}, nm[b]	IR, V_{max} (KBr) (cm^{-1})	Chemical shifts (δ) from Me_4Si[c] 1H	1'H	2'H	3'H	4'H	5'H	substituent H
1	338(14000)	3413, 3120, 2939, 1697, 1604,	7.7	3.4 dd	3.9	4.1	4.6	5.6	
	252	1419, 1211, 1037	s	3.6 dd	m	dd	m	d	
2	315(8012)	3394, 2927, 1554, 1519, 1473,	7.7	3.45dd	3.9	4.1	4.65	5.6	2.4(3H, s)
	242	1238, 1126, 1053	s	3.6 dd	m	dd	m	d	
3	318(8000)	3417, 2927, 1554, 1519, 1419,	7.7	3.4 dd	3.9	4.1	4.6	5.6	1.25(3H, t), 3(2H, q)
	245	1126, 1053	s	3.6 dd	m	dd	m	d	
4	302	3201, 2955, 1685, 1654, 1569,	7.7	3.45dd	3.9	4.1	4.6	5.6	3.85(2H, t),
	247	1388, 1377, 1083	s	3.60dd	m	dd	m	d	2.70(2H, t)
5	301(9000)	3406, 2922, 1577, 1419, 1126,	7.7	3.45dd	3.9	4.1	4.6	5.6	2.3(3H, s), 7.2(2H, d)
	252	1045	s	3.6 dd	m	dd	m	d	7.4(2H, d)
6	323	3398, 3001, 1582, 1499, 1387,	7.8	3.45dd	3.90	4.1	4.6	5.6	7.30(5H, m)
	259	1132, 1089	s	3.60dd	m	dd	m	d	4.01(2H, s)
7	282(1547)	3421, 2981, 1620, 1577, 1438,	7.98	3.78dd	4.2	4.35	4.7	5.9	4.05(3H, s)
	243	1377, 1342, 1080	s	3.9 dd	m	dd	m	d	
8	283	3421, 2985, 1697, 1577, 1434,	7.98	3.7 dd	4.2	4.3	4.7	5.9	3.97(2H, t),
	242	1342, 1083	s	3.9 dd	m	dd	m	d	1.38(3H, q)
9	321	3421, 2927, 1650, 1573, 1558,	7.70	3.45dd	3.90	4.15	4.70	5.90	
	243	1450, 1064	s	3.65dd	m	dd	m	d	
10	292(11000)	3344, 3109, 2939, 1634, 1620,	[d]7.97	3.56dd	3.98	4.11	4.50	5.67	
	248	1531, 1400, 1087	s	3.66dd	m	dd	m	d	
11	[a]281(7600)	3224, 3120, 2923, 2279, 1627,	7.95	3.5 dd	3.98	4.18	4.45	5.67	2.95(3H, br s)
	[a]248	1593, 1496, 1311, 1107	s	3.6 dd	m	dd	m	d	
12	278(10800)	3427, 3101, 2927, 1639, 1569,	7.95	3.5 dd	3.98	4.18	4.45	5.67	3.41(6H, br s)
	250	1400, 1342, 1276, 1087	s	3.6 dd	m	dd	m	d	
13	294	3259, 3116, 2993, 1670, 1643,	7.90	3.50dd	3.90	4.15	4.50	5.56	3.10(1H, sep)
	248	1600, 1573, 1400, 1118, 1068	s	3.65dd	m	dd	m	d	1.20(6H, s)
14	299(11800)	3336, 2936, 1643, 1600, 1558,	[d]8.0	3.81dd	4.25	4.4	4.7	5.89	3.6(2H, m), 1.3(3H, t)
	251	1519, 1458, 1404	s	3.9 dd	m	dd	m	d	
15	294	3290, 2960, 1647, 1600, 1558,	7.95	3.50dd	3.98	4.16	4.45	5.67	1.23(3H, t), 1.5(2H, m)
	247	1458, 1126, 1059	s	3.65dd	m	dd	m	d	3.30(2H, m)
16	295(9601)	3271, 3136, 2958, 1650, 1558,	7.98	3.55dd	3.9	4.15	4.50	5.65	0.92(3H, t), 1.3(2H, m)
	249	1404, 1245, 1122, 1083	s	3.65dd	m	dd	m	d	1.55(2H, d), 3.35(2H)
17	296(9400)	3260, 3125, 2936, 1648, 1559,	7.98	3.55dd	3.9	4.1	4.50	5.65	0.90(3H, t)
	248	1450, 1128, 1078	s	3.65dd	m	dd	m	d	1.20-1.70(6H, m)
									3.32(2H, d)
18	296(9671)	3255, 3128, 2931, 2854, 1639,	7.95	3.50dd	3.90	4.15	4.50	5.56	1.21(2H, t), 1.3(4H, m)
	249	1600, 1519, 1400, 108	s	3.65dd	m	dd	m	d	1.52-2.0(4H, m)
19	298(9011)	3294, 3201, 2952, 1643, 1650,	7.90	3.50dd	3.90	4.5	4.50	5.56	2.99(2H, t), 2.90(2H, t)
	249	1519, 1458, 1130, 1050	s	3.65dd	m	dd	m	d	
20	293(8734)	3388, 2939, 1650, 1635, 1558,	8.01	3.50dd	3.90	4.15	4.50	5.56	3.61(2H, t), 2.68(2H, t)
	248	1539, 1080	s	3.65dd	m	dd	m	d	
21	[a]301(22900)	3290, 2928, 1650, 1592, 1455,	8.19	3.58dd	3.97	4.14	4.51	5.20	7.03(1H, t), 7.31(2H, m)
	240	1405, 1201, 1028	s	3.69dd	m	dd	m	d	7.96(2H, d)
22	293(10077)	3271, 2927, 1639, 1558, 1454,	8.01	3.58dd	3.91	4.15	4.51	5.50	4.05(2H, t), 7.25(2H, t)
	247	1404, 1242, 1083	s	3.69dd	m	dd	m	d	7.3(3H, d)
23	295(8900)	3295, 2999, 1659, 1635, 1550,	8.01	3.50dd	3.97	4.15	4.51	5.50	7.5(1H, s), 6.4(1H, t)
	249	1400, 1083		3.69dd					6.3(1H, s), 4.8(2H, br)

[a]Determined in 95% MeOH, [b]Determined in H_2O, [c]Solvents: DMSO-d_6, [d]Solvents: D_2O

as cyclohexyl amines, phenyl amine and benzyl amine in high yield. When the intermediate IV with benzyl amine in ethanol solution was refluxed for 5 hrs and the product was then treated with methanolic ammonia, 6-N-benzyl isoguanosine was obtained and isolated as a crystalline solid in 75% yield. In this studies, we followed reaction progress by thin layer chromatography. As reaction goes on, we detected increase of toluenethiol in front-line of TLC-chromatogram that was removed from intermediate IV. Separated toluenethiol was readily oxidized to disulfide that could be discarded by trituration several times with n-hexane. Structure elucidation of all synthetic compounds were accomplished with ^{1}H-NMR and IR spectrometer and all synthetic compounds have same ribose sugar moiety and then we finally confirmed structure of syn-

Table III. Cytotoxicity of various 6-substituted-2-oxo-purine nucleosides against Pogras 1, Hela and Molt-4 tumor cell-lines

Compounds	ED_{50} (μg/ml)		
	Pogras 1	Hela	Molt-4
1	70	60	80
2	30	100	>100
3	35	100	>100
4	>200	–[a]	100
5	>200	90	50
6	>200	–	90
7	>200	–	–
8	>200	–	–
9	100	80	88
10	34	70	15
11	70	70	100
12	100	150	>100
13	125	100	70
14	>200	>200	>200
15	>200	>200	100
16	>200	>200	100
17	>200	–	>200
18	38	100	100
19	>200	–	>400
20	>200	100	200
21	>200	100	>200
22	>100	100	–
23	50	98	70

[a]Not examined

thetic compounds with each ^{1}H-NMR spectra of N^6-substituted group (Table II).

Biology. Antiproliferative Activity *in vitro*

Growth inhibition of Pogras 1, Hela and Molt-4 tumor cell-lines in culture by 6-substituted-2-oxo-purine nucleosides was shown in Table III. Cytotoxicity of 6-substituted-2-oxo-purine nucleosides was relatively low against tested tumor cell-lines. ED_{50} values of these compounds were above 100 μg/ml except compounds including amino, sulfur and furfurylamine in C-6 purine base. ED_{50} value of most effective compound 10, isoguanosine, was in the range of 10-70 μg/ml against all tumor cell-lines. From the result of structure-activity relationship studies conducted so far, we report that: (1) NH_2 or SH group in C-6 purine base is essential for high cytotoxicity against various tumor cell-lines. (2) When 6-amino hydrogens in isoguanosine substituted by non-polar alkyl group, the cytotoxicity always diminishes, which is contrary to the cases of adenosine derivatives reported in the literatures (Fleysher, 1972).

EXPERIMENTAL

General Experimental Procedure

Guanosine was purchased from Sigma Chemical Co. and all other reagents from Aldrich Chemical Co.. Compounds 10, 11, 12 and 21 were synthesized by Divarkar's method (Divarkar *et al.*, 1991). All melting points are uncorrected. IR spectra were taken on a Shimadzu IR-435. NMR spectra were obtained on a Jeol 280 FT-NMR spectrometer with TMS as the chemical shift standard. UV-VIS spectrophotometer were obtained on a Kontron 860. Merck silica gel 60H was used for short column chromatography; Merck silica gel 60 F_{254} TLC plates were developed in a mixture of butanol, acetic acid and water (4 : 1 : 5 v/v).

6-Mercapto-2-Hydroxy-9-β-D-Ribofuranosyl Purine (1)

Starting material (0.3 g) was dissolved in a solution of ethanol (5 ml) and to this was added H_2O (5 ml) saturated H_2S at O℃. The resulting solution was heated at 70℃ in tightly closed cap vial for 24 hrs and then concentrated under reduced pressure and ethanol (5 ml) was added. The resulting mixture was re-evaporated under reduced pressure, and this process was repeated twice more. The residue was dissolved in methanolic ammonia and stood room temp. for 12 hrs. And it was concentrated under reduced pressure and triturated several times with n-hexane and then was dissolved in hot ethanol. The resulting precipitate was filtered and recrystallized from a small amount of ethanol to afford 0.071 g (yield, 42%) of light yellow product.

Similarly, according to the above-mentioned method, compounds 2, 3, 4, 5 and 6 were prepared and their experimental conditions and spectral data are given Table I, II.

6-Methoxy-2-Hydroxy-9-β-D-Ribofuranosyl Purine (7)

A solution of compound IV (0.3 g) in methanolic methoxide was refluxed for 12 hrs. The solution was brought with dilute HCl to pH 7 and concenturated in vacuo to dryness. The result oil was crystallized from a small amount of ethanol to give 0.15 g of title compound 7 (yield, 91%) of white crystal.

6-Ethoxy-2-Hydroxy-9-β-D-Ribofuranosyl Purine (8)

The title compound was prepared in a similar manner to that described above for compound 7 by reacting key intermediate IV and sodium ethoxide in methanol (yield, 41%).

6-Cyano-2-Hydroxy-9-β-Ribofuranosyl Purine (9)

Starting material IV 0.5 g was dissolved in 8 ml of acetonitrile and to this was added NaCN (0.1 g) dissolved in H_2O (2 ml). The resulting solution was heated at 60℃ for 7 hrs. The reactant was evaporated under reduced pressure. The resulting oil was dissolved in

methanolic ammonia and kept at room temperature for 24 hrs and evaporated under reduced pressure. Resulting precipitate was recrystallized from small amount of methanol to afford 0.07 g of compound 9 (yield, 42%).

Isoguanosine (10)

Conc. aq. ammonia (9 ml) was added to a solution of compound 10 (0.35 g, 0.68 mmol) in ethanol (3 ml). The resulting solution was heated at 70℃ in Pierce Reacti-vials for 17 hrs. The products were concentrated under reduced pressure and then triturated serveral times with diethyl ether and then dissolved in a hot mixture of ethanol (12.5 ml) and water (12.5 ml). The resulting solution was heated with activated charcoal under reflux for 5 mins, and was then cooled, and filtered through celite. Concentration of the filterate gave 0.141g of isoguanosine (yield, 73%) as a solid.

6-N-Methylisoguanosine (11)

Compound (1 g) was dissolved in a 33% solution of methylamine in ethanol (18 ml) and the resulting solution was stirred at room temperature for 3 hrs. The products were concentrated under reduced pressure and the residue was triturated several times with diethyl ether. The residual solid was crystallized from ethanol to give 0.52 g of the title compound 11 (yield, 90%).

The title compounds 11-20 were prepared in a similar manner to that described above for compound 10 by reacting key intermediate IV and appropriate amines. The result products and their spectroscopic data are shown in Table I, II.

6-N-Phenyl Isoguanosine (21)

Key intermediate IV (0.3 g) and freshly distilled aniline (0.1 ml) were refluxed in anhydrous pyridine (8 ml) solution under nitrogen for 40 hrs. The products were then concentrated under reduced pressure and the residue was fractionated by short column chromatography on silica gel to give oily product. The residue was dissolved in methanolic ammonia, and stood at room temp. for 12 hrs. It was concentrated under reduced pressure and triturated several times with n-hexane. Crystallization of the solid residue from ethanol gave 0.11 g of the title compound 20 (yield, 54%).

6-N-Benzyl Isoguanosine (22) and 6-N-Furfuryl Isoguanosine (23)

The title compounds 22 and 23 were prepared by the same method as for 11 from compound IV and benzyl amine and furfuryl amine instead of methyl amine. The each yield is 75% and 52%, respectively.

Cytotoxicity Against Some Tumor Cell-Lines *in vitro*

For in vitro studies, tumor cell-lines Molt-4 and Hela were purchased from ATCC (American Tissue Culture Collection) and Pogras-1 from Israel Weizman Institute. Tumor cell-lines were grown in RPMI-1640 medium supplemented with 10% FBS, streptomycin 0.1 mg/ml and penicillin 100 units/ml at 37℃. After incubation for 48 hrs at 37℃ in 5% carbon dioxide, the number of remaining cells was counted with a cell counter by MTT method (Mosmann, 1983) and cytotoxicities of isoguanosine against each tumor cell-lines were evaluated in terms of the ED_{50}. These results are shown in Table III.

REFERENCES CITED

Akira, Y., Akira, K. and Tohru, U., Reaction of 6-methylsulfonylpurine riboside with carbon nucleophiles and the synthesis of 6-alkylpurine nucleosides. *Chem. Pharm. Bull.*, 28(1), 150-156 (1980).

Akihiro, Y. and Masaru, O., Cyclization of 5-amino-1-β-D-ribofuranosyl- imidazole-4-carboxamide (AICA-riboside): a review. *J. Heterocyclic Chem.*, 15, 353-358 (1978).

Davoll, J. and Bertram, A. L., Some synthetic analogs of the natural purine nucleosides. *J. Am. Chem. Soc.*, 73, 3174-3176 (1951).

Divakar, K. J., Mina, M., Colin, B. R., Yogesh, S. S. and Karl, A. D. S., Conversion of guanosine into isoguanosine and derivatives. *J. Chem. Soc.*, PERKIN TRANS 1, 771-774 (1991).

Fleysher, M. M., N^6-substituted Adenosine: synthesis, biological activity, and some structure-activity relationship. *J. Med. Chem.*, 15(2), 187-191 (1972).

Fox, J. J., Wempen, I., Hampton and Doerr, L. L., Thiation of nucleosides. 1. synthesis of 2-amino-6-mercapto-9-β-D-ribofuranosylpurine (thioguanosine) and related purine nucleosides. *J. Am. Chem. Soc.*, 80, 1669 (1958).

Frederik, A. F., Geraldine, J. F., Ronald, J. N. and Harry, S. M., Isoguanosine: isolation from an animal. *Science*, 212(1), 557-558 (1981).

Kazunobu, M. and Tohru, U., Nucleosides and nucleotides XIII, synthesis of thiopurine nucleosides from adenosine and guanosine derivatives by the sulfhydrolysis. *Chem. Pharm. Bull.*, 23(9), 2064-2069 (1975).

Kim, J. H., Lee, S. J., Han, Y. B., Moon, J. J. and Kim, J. B., Isoguanosine; isolation and anti-tumor activity studies. *Arch. Pharm. Res.*, 17(2), 115-118 (1994).

Mosmman, T., Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. *J. Immunol. Method*, 65, 55-63 (1983).

Nair, V. and Toung, D. A., A new synthesis of isoguanosine. *J. Org. Chem.*, 50, 406 (1985).