Potential Antitumor α-Methylene-γ-butyrolactone-Bearing Nucleic Acid Base. 3. Synthesis of 5'-Methyl-5'-[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans

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Search for a new α-methylene-γ-butyrolactone-bearing 6-substituted purine as a potental antitumor agent has led to synthesize seven, hitherto unreported, 5'-Methyl-5'-[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (H, Cl, I, CH_3, NH_2, SH, >C=O) (**6a-g**). These include 5'-Methyl-5'-[(9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6a**), 5'-Methyl-5'-[(6-chloro-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6b**), 5'-Methyl-5'-[(6-iodo-9H-purin-9-yl) methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6c**), 5'-Methyl-5'-[(6-methyl-9H-purin-9-yl) methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6d**), 5'-Methyl-5'-[(9H-adenin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6e**), 5'-Methyl-5'-[(6-mercapto-9H-purin-9-yl) methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6f**) and 5'-Methyl-5'-[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (**6g**) which were made by the Reformatsky-type reaction of ethyl α-(bromomethyl) acrylate with the corresponding (6-substituted-9H-purin-9-yl)-2-propanone intermediates (**5a-g**). These ketone intermediates **5a-g**, 1-(9H-purin-9-yl)-2-propanone (**5a**), 1-(6-chloro-9H-purin-9-yl)-2-propanone (**5b**), 1-(6-iodo-9H-purin-9-yl)-2-propanone (**5c**), 1-(6-methyl-9H-purin-9-yl)-2-propanone (**5d**), 1-(9H-adenin-9-yl)-2-propanone (**5e**), 1-(6-mercapto-9H-purin-9-yl)-2-propanone (**5f**), and 1-(9H-hypoxanthin-9-yl)-2-propanone (**5g**) were directly obtained by the alkylation of the 6-substituted purine bases with the chloroacetone in the presence of K_2CO_3 (or NaH) under DMF (or DMSO).The preliminary in vitro cytotoxcity assay for the synthetic α-methylene-γ-butyro-lactone compounds (**6a-g**) were determined against three cell lines (PM-3A, P-388, and K-562) and showed the moderate antitumor activity (IC_{50} ranged from 1.4 to 4.3 μg/ml) with the compound 5'-methyl-5'-[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (**6g**) showing the least antitumor activity.

Key words : 5'-Methyl-5'-[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran, 6-Substituted-9H-purin-9-yl-2-propanone, Cytotoxic moiety, Antitumor activity, IC_{50}, Reformatsky reaction, Human chronic myelogenous (K-562), Mouse lymphoid neoplasma (P-388), Mouse mammary carcinoma (FM-3A)

INTRODUCTION

The α-methylene-γ-butyrolactone ring is an integral building block of many natural products, especially the sesquiterpene lactones, which exhibit interesting biological properties (Grieco,1975). Structure-activity relationships for these complex natural products have indicated that one of the structural requirements for significant cytotoxic antitumor activity is an $-CH_2=C$ $-C=O$ moiety as part of an ester as well as a ketone, present in elephantophin (**1**) (Kupchan, *et al.*, 1969a), tenulin (**2**) (Hall *et al.*, 1977), helenalin (**3**) (Hall *et al.*, 1977), and vernolephin (**4**) (Kupchan *et al.*, 1969b)

Kupchan has demonstrated that α-methylene-γ-lactone system can act as the alkylating center for cytotoxic antitumor lactones (for example **1**, **2**, **3** and **4**). A Michael-like reaction between biological cellular nucleophiles such as L-cysteine, glulathione or thiol-rich enzymes (phosphofructokinase, glycogen synthetase and DNA polymerase), can act to the α-methylene-γ-butyrolactone moiety itself (Lee *et al.*, 1975; Kupchan *et al.* 1970). It has been established that the

1   2   3   4
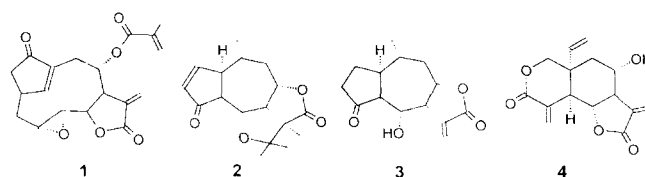
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α-methylene-γ-butyrolactone is the most reactive chemical functionality in both **1**, **3** and **4**, with no reaction being observed between L-cysteine and the epoxide **1** or the endocyclic α,β-unsaturated lactone in **1**. These views are in accord with the theory of tumor inhibition by the selective alkylation of biological macromolecules which have been advanced by Kupchan and coworker.

A large number of possible drug candidates bearing this functionality of the general structure of α-methylene-γ-butyrolactone moiety have been synthesized (Lee et al., 1975; Dehal et al., 1980; Heindel et al., 1981; Cassady et al., 1978; Rosowsky et al., 1974; Sanyal et al., 1986), with a view to develop effective clinical drugs since naturally found derivatives have therapeutic indices that prelude their clinical use. Several new synthetic approaches to the development of such a cytotoxic α-methylene-γ-butyrolactone moiety are excellently reviewed (Ohler et al., 1970; Grieco, 1975; Gammill et al., 1975).

As part of our effort to develop more useful antitumor agents (Kim et al., 1992; 199/3a, b; 1994a, b; 1995; 1996), we were particularly interested in synthesizing suitably substituted nucleic acid base-bearing this moiety as a biological carrier. An extensive literature survey revealed that relatively scanty literature references are known. We have synthesized potential target-specific alkylating agents by introducing the antitumor cytotoxic moiety, α-methylene-γ-butyrolactone function into 6-substituted purine nucleic acid bases **5a-g**, and evaluated these synthesized compounds, **6a-g** against three cell lines (K-562/S, P-388/S and FM-3A/S).

MATERIALS AND METHODS

Melting points were determined on an electrothermal capillary melting point apparatus and Haake Buchler Melting point apparatus and uncorrected. TLC was performed on glass plates coated with silicone oxide (silica gel 60F$_{254}$) and compounds were visualized using a UV lamp. Proton nuclear magnetic resonance and ^{13}C-NMR spectra were obtained with a Varian EM-360 spectrophotometer and Varian Gemmini 200 MHz, Brucker AM 300 and DPX 200 (solution in dimethylsulfoxide-d6 with tetramethylsilane as internal standard). The organic solvents and chemicals were obtained from commercial products and purified by the appropriate methods before use. Pertinent data for synthesized compounds (**5a-g**, **6a-g**) are listed in Table I.

General procedure for the synthesis of 1-[(6-substituted-9H-Purin-9-yl)]-2-propanone (5a-g).

To a dissolved solution of purine (2.02 g, 16.65 mmol) in DMF (50 ml) was added K$_2$CO$_3$ (2.32 g, 16.65 mmol). The mixture was stirred at room temperature for 30 min by adding a dissolved chloroacetone (1.5 ml, 18.32 mmol) in DMF (15 ml) in small portions during 1-2 hour period. The reaction mixture was continuously stirred for additional 18 hours, and filtered through celite, and the filtrate was evaporated in vaccuo. The residue was dissolved in CHCl$_3$ and washed with aq NaCl solution, and 10% NaHCO$_3$. The organic layers were dried over anhyd. MgSO$_4$, filtered and evaporated, and the residues were applied to flash column chromatography (CH$_2$Cl$_2$:MeOH=10:1) to obtain 9-alkylated product along with a minor amount of 7-alkylated isomer (0.9 g, 30% yield).

1-(9H-Purine-9-yl)-2-propanone (5a): mp: 151~153 °C; 1.9 g (65% yield); IR (KBr): 3119, 2960, 1727, 1600, 1582, 1505, 1410, 1356, 1205, 1190 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 2.36 (s, 3H), 5.14 (s, 2H), 8.09 (s, 1H), 8.96 (s, 1H), 9.17 (s, 1H); Mass (m/e): 176 (M$^+$), 149, 134, 120, 106, 97, 86.

7-Alkylated isomer, 1-(9H-purine-7-yl)-2-propanone: mp. 216~217°C; IR (KBr): 3060, 2978, 1724, 1607, 1521, 1487, 1421, 1372, 1277, 1178 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 2.28 (s, 3H), 5.46 (s, 2H), 8.52 (s, 1H),

Table I. IC$_{50}$ values for α-methylene-γ-butyrolactone-bearing 6-substituted purines as determined by MTT assay method

Comp. NO.	IC$_{50}$[a]			Comp. NO.	IC$_{50}$[a]		
	FM-3A[b]	P-388[c]	K-562[d]		FM-3A[b]	P-388[c]	K-562[d]
6a	3.1	2.2	2.4	**5a**	>100	20	68
6b	2.6	2.3	2.7	**5b**	32	23	34
6c	2.1	3.4	2.6	**5c**	12	14	5.2
6d	3.2	3.0	1.4	**5d**	17	>100	38
6e	>10	>6.7	>2.8	**5e**	82	44	70
6f	4.3	2.0	2.6	**5f**	>100	>100	>100
6g	>100	>100	>100	**5g**	>100	65	15

[a]Mean values of triplicate runs. The concentration of synthesized compounds required to reduce cell numbers to 50% of controls in a growth inhibition assay.
[b]Mouse mammary carcinoma cell.
[c]Mouse lympoid neoplasma.
[d]Human chronic meylogenous leukemia cell.
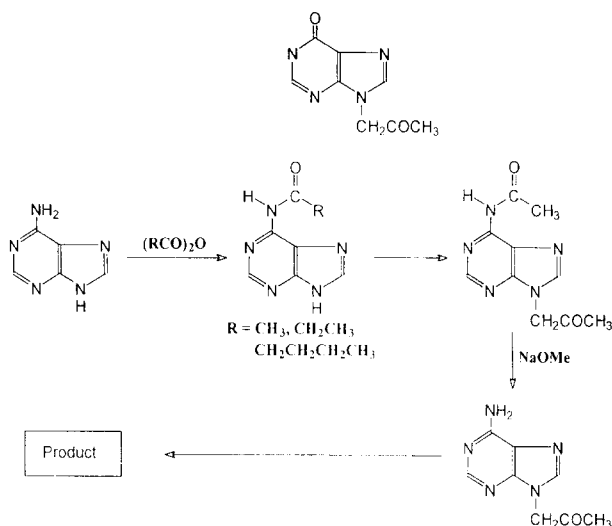
8.98 (s, 1H), 9.90 (s, 1H); Mass (m/e): 176 (M⁺), 149, 134, 120, 106, 97, 86.

1-[(6-Chloro-9H-purin-9-yl)]-2-propanone (5b): mp: 168~171°C; 62% yield IR (KBr): 3109, 2969, 1726, 1593, 1583, 1502, 1437, 1336, 1259, 1207, 1151 cm⁻¹; ¹H NMR (CDCl₃): δ 2.37 (s, 3H), 5.14 (s, 2H), 8.11 (s, 1H), 8.72 (s, 1H); Mass (m/e): 210 (M⁺), 168, 149, 114, 104, 97, 84.

1-[(6-Chloro-9H-purin-7-yl)]-2-propanone: mp: 182~184°C; 22% yield; IR (KBr): 3065, 2981, 1731, 1604, 1542, 1483, 1365, 1283, 1174, 976 cm⁻¹; ¹H NMR (CDCl₃): δ 2.38 (s, 3H), 5.31 (s, 2H), 8.15 (s, 1H), 8.90 (s, 1H); Mass (m/e): 210 (M⁺), 168, 149, 140, 113, 97, 84.

1-[(6-Iodo-9H-purin-9-yl)]-2-propanone (5c): mp: 167~169°C; 85% yield; IR (KBr): 3117, 2963, 1731, 1583, 1554, 1493, 1400, 1327, 1173, 918, 776 cm⁻¹; ¹H NMR (CDCl₃): δ 2.36 (s, 3H), 5.11 (s, 2H), 8.11 (s, 1H), 8.60 (s, 1H); Mass (m/e): 302 (M⁺), 279, 260, 215, 167, 149, 133, 119, 105.

1-[(6-Methyl-9H-purin-9-yl)]-2-propanone (5d): mp: 137~139°C; 86% yield; IR (KBr): 3103, 2959, 1730, 1594, 1426, 1332, 1223, 1187, 1031, 903, 795 cm⁻¹; ¹H NMR (CDCl₃): δ 2.34 (s, 3H), 2.88 (s, 2H), 5.10 (s, 1H), 8.01 (s, 1H), 8.82 (s, 1H); Mass (m/e): 190 (M⁺), 162, 147, 134, 120, 105, 94.

1-(9H-Adenin-9-yl)-2-propanone (5e): White solid; 94% yield; mp: 252~253°C; IR (KBr): 3349, 3174, 2911, 1723, 1659, 1600, 1579, 1488, 1425, 1331, 1249, 1179, 1075, 793 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.23 (s, 3H), 5.15 (s, 2H), 7.19 (brs, 2H), 7.98 (s, 1H), 8.10 (s, 1H); Mass (m/e): 191 (M⁺), 167, 149, 137, 127, 111, 97.

1-(9H-Hypoxanthin-9-yl)-2-propanone (5g): To a dissolved mixture of 1-(9H-Adenine-9-yl)-2-propanone (5e) (573 mg, 3 mmol) in distilled water (72 ml) was added NaNO₂ (1.8 g, 26 mmol) and acetic acid (2.4 ml, 42 mmol) and stirred at room temperature for 12

hrs. The whole reaction mixture was neutralized with 10% NaHCO₃ and evaporated *in vaccuo*. The residues were washed with CH₃OH several times (3×15 ml) and filtered. The filtrates were evaporated in vaccuo, and applied to flash column chromatography (CHCl₃: CH₃OH=8:2) to afford white solid, 60% yield; mp: 280~282°C (lit. 282~284°C); IR (KBr): 3432, 3052, 2974, 1706, 1592, 1562, 1421, 1344, 1208, 1182, 1045 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.23 (s, 3H), 3.30 (s, 1H), 5.17 (s, 2H), 7.91 (s, 1H), 7.99 (s, 1H); Mass (m/e): 191 (M⁺), 150, 127, 111, 97.

General procedure for the synthesis of ethyl α-(bromomethyl)acrylate

To a solution of dry, distilled ethylene glycol (12.6 g, 2.3 mol) in dry THF (43 ml) was added cautiously NaH (3.0 g, 0.12 mol). After the first vigorous reaction had subsided, the mixture was heated under reflux for 24 hours. The syrupy suspension of sodium ethylene glycolate was cooled to room temperature and then added slowly to a solution of ethyl β,β' -dibromoisobutyrate (27.8 g, 0.10 mol) in THF (85 ml). The temperature was kept below 45°C by controlling the rate of reactions when addition was complete and the mixture was stirred for 90 minutes and then poured into water (400 ml). The organic layer which separated was extracted into CH₂Cl₂ (100 ml × 3). The aqueous layer was acidified with aqueous 5N nitric acid and treated with silver nitrate. After drying, the silver bromide precipitate weighed 10 g (60%). The methylene chloride was evaporated under reduced pressure and the residue was fractionated through the column, bp 44~45°C (1.7 mmHg).

N⁶-Propionyl adenine

A mixture of adenine (1.5 g, 0.01 mol) and propionic anhydride (10 ml, 1 mol) was refluxed for 1 hr under the temperature of 160°C, and cooled to 0°C, and added excess of diethylether-hexane (1:1). The solids were separated, and crystallized to obtain white solid, 82% yield; mp: 218~220°C; IR (KBr): 3267, 3104, 2910, 1691, 1546, 1392, 1370, 1307, 1208, 1072 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.13 (t, 3H, J=7.5 Hz) 2.57 (q, 2H, J=7.5 Hz), 8.39 (s, 1H), 8.61 (s, 1H), 11.03 (brs, 1H).

N⁶-Acetyl adenine: White solid; 85% yield; mp:> 290°C (dec.) (lit. 280~285°C); IR (KBr): 3274, 3103, 1687, 1541, 1453, 1398, 1222, 1119, 1050, 948, 878 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.24 (s, 3H), 8.39 (s, 1H), 8.61 (s, 1H), 11.12 (brs, 2H).

1-(N⁶-protected-9H-adenin-9-yl)-2-propanone: Slight yellow solid; 85% yield; mp: 200~202°C (dec.) (lit. 200~201°C); IR (KBr): 3432, 3268, 2930, 1723, 1685, 1611, 1586, 1461, 1362, 1232, 1175, 1042, 797 cm⁻¹; ¹H NMR (DMSO-d₆): δ 2.27 (s, 6H), 5.29 (s, 2H),

8.29 (s, 1H), 8.59 (s, 1H), 10.52 (s, 1H); Mass (m/e): 233 (M$^+$), 191, 149, 141, 127, 111, 97.

General procedure for the synthesis of 5'-methyl-5'-[(6-substituted-9H-purine-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofurans (6a-g)

A solution of ethyl α-(bromomethyl)acrylate (1.16 g, 6 mmol) in anhydrous THF (30 ml) was added dropwise with vigorous stirring under nitrogen to a mixture of granulated active Zn (490 mg, 7.5 mmol), p-hydroquinone (8 mg) and 1-[(6-substi- tuted-9H-purin)-9-yl)]-2-propanone (5a-d) (528 mg, 3 mmol) in anhydrous THF (20 ml). Once the reaction has started, addition was adjusted such that the temperature does not rise above 40~50°C. The reaction mixture was stirred for 4 hrs at 50°C, cooled, and poured into ice-cold 10% HCl (25 ml). The reaction mixture was extracted with CHCl$_3$ (4×100 ml), and the CHCl$_3$ extractions were washed with aq. NaHCO$_3$, followed by aq NaCl dried over anhyd. MgSO$_4$. Filtration and evaporation in vaccuo gave residues which were applied to flash column chromatography (CH$_2$Cl$_2$:MeOH= 15:1) to afford the desired products in good yields.

5'-Methyl-5'-[(9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6a): Slight yellow solid; 13% yield; mp: 148~150°C; IR (KBr): 3130, 3089, 2982, 1763, 1663, 1593, 1501, 1406, 1250, 1253, 1106, 1067 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.51 (s, 3H), 2.77~3.08 (m, 2H), 4.52 (t, 2H, J=5.9 Hz), 5.39 (d further splitted t, 1H, J=2.5 Hz), 6.02 (d further splitted t, 1H, J=2.8 Hz), 8.21 (s, 1H), 8.99 (s, 1H), 9.16 (s, 1H); ^{13}C NMR (DMSO-d$_6$): δ 25.2, 37.7, 51.3, 83.1, 116.5, 122.3, 133. 9, 135.6, 148.4, 148.9, 153.1, 169.4; Mass (m/e) : 244 (M+), 228, 200, 185, 157, 134, 129, 111, 97.

5'-Methyl-5'-[(6-chloro-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofu ran (6b): White solid; 58 % yield; mp: 150~152°C; IR (KBr): 3114, 2982, 1765, 1662, 1580, 1439, 1337, 1267, 1129, 1068 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.49 (s, 3H), 2.78~3.02 (m, 2H), 4.50 (s, 2H), 5.46 (d further splitted t, 1H, J=2.4 Hz), 6.10 (d further splitted t, 1H, J=2.8 Hz), 8.23 (s, 1H), 8.76 (s, 1H); ^{13}C NMR (DMSO-d$_6$): δ 25.1, 37.6, 51.8, 82.9, 122.5, 131.2, 135.6, 148.9, 150.1, 152.7, 153.3, 169.3; Mass (m/e): 278 (M$^+$), 237, 217, 169, 155, 131, 119, 109.

5'-Methyl-5'-[(6-iodo-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6c): White solid; 22% yield; mp: 149~151°C; IR (KBr): 3113, 2980, 1765, 1590, 1436, 1267, 1134, 1067, 939, 656 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.49 (s, 3H), 2.79~3.02 (m, 2H), 4.51 (s, 2H), 5.47 (d further splitted t, 1H, J=2.4 Hz), 6.10 (d further splitted t, 1H, J=2.8 Hz), 8.24 (s, 1H), 8.76 (s, 1H); 13C NMR (DMSO-d$_6$): δ 25.1, 37.6, 51.9, 82.9, 122.5, 131.2, 135.6, 148.9, 150.1, 152.7, 153.3, 169.4; Mass (m/e): 370 (M$^+$), 327, 279, 168, 136, 127, 111.

5'-Methyl-5'-[(6-methyl-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofu ran (6d): White soli; 80 % yield; mp: 113~115°C; IR (KBr): 3088, 2325, 1762, 1659, 1595, 1422, 1332, 1068, 944, 812, 707 cm^{-1}; ^{1}H NMR (DMSO-d^6): δ 1.50 (s, 3H), 2.74~2.82 (m, 1H), 2.87 (s, 3H), 2.98~3.05 (m, 1H), 4.47 (d, 2H, J= 6.6 Hz), 5.39 (d further splitted t, 1H, J=2.5 Hz), 6.04 (d further splitted t, 1H, J=2.8 Hz), 8.11 (s, 1H), 8.84 (s, 1H); ^{13}C NMR (DMSO-d$_6$): δ 19.9, 25.3, 37.4, 51.2, 82.2, 123.5, 132.8, 134.0, 145.0, 151.3, 152.9, 160.2, 168.9; Mass (m/e): 258 (M$^+$), 149, 135, 129, 111, 97.

5'-Methyl-5'-[(9H-adenin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6e): White solid; 62% yield; mp. 246~248°C; IR (KBr): 3341, 3161, 1760, 1655, 1601, 1579, 1487, 1309, 1272, 1114, 1064 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.42 (s, 3H), 2.76~3.18 (m, 2H), 4.41 (d, 2H, J=3.8 Hz), 5.43 (d further splitted t, 1H, J =2.4 Hz), 5.77 (d further splitted t, 1H, J=2.8 Hz), 7.20 (brs, 2H), 7.95 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (DMSO-d$_6$): δ 24.4, 50.2, 82.2, 121.3, 130.1, 134.1, 134.8, 141.3, 149.2, 149.9, 152.6, 156.0, 168.6; Mass (m/e): 259(M$^+$), 215, 183, 149, 135, 127.

5'-Methyl-5'-[(6-mercapto-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6f): To a dissolved solution of 5'-Methyl-5'-[(6-chloro-9H-purin-9-yl) methyl]-2'-oxo-3'-methylenetetrahydrofuran (6b) (168 mg, 0.6 mmol) in anhydrous 1-propanol (6 ml) was added thiourea (52 mg, 0.68 mmol)and the mixture was refluxed for 1 hr, and cooled to 0°C. The solid formed, was filtered, and washed with 1-propanol, crystallization gave a slight yellow solid; 93 mg 55% yield; mp: 245~248°C; IR (KBr): 3108, 2983, 1762, 1593, 1407, 1336, 1275, 1192, 1067, 952 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.43 (s, 3H), 2.80~3.17 (m, 2H), 4.47 (s, 2H), 5.56 (d further splitted t, 1H, J=2.3 Hz), 5.85 (d further splitted t, 1H, J=2.7 Hz), 8.15 (s, 1H), 8.24 (s, 1H), 13.76 (brs, 1H); ^{13}C NMR (DMSO-d$_6$): δ 25.2, 37.5, 51.5, 82.8, 122.6, 135.3, 135.5, 144.5, 145.4, 146.0, 169.4, 176.8; Mass (m/e): 276 (M$^+$), 250, 206, 150, 141.

5'-Methyl-5'-[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6g): White solid; 31% yield; mp: 272~274°C (lit. 268~270°C); IR (KBr): 2978, 1759, 1685, 1556, 1520, 1465, 1222 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 1.42 (s, 3H), 2.97 (m, 2H), 4.43 (m, 2H), 5.51 (d further splitted t, 1H, J=2.3 Hz), 5.82 (d further splitted t, 1H, J=2.8 Hz), 7.94 (s, 1H), 8.08 (s, 1H); ^{13}C NMR (DMSO-d$_6$): δ 25.2, 37.4, 51.4, 82.9, 122.4, 124.3, 135.6, 141.8, 146.6, 149.7, 157.5, 169.4; Mass (m/e): 260 (M$^+$), 183, 167, 149, 127, 111.

MTT-microculture tetrazolium assay

The antitumor effect of the synthesized compounds was determined by the modified methods (Mosmann et al., 1983; Carmichael et al., 1987; Kim et al., 1994b,

c, d). The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co., St. Louis, M.O.) by the mitochondrial dehydrogenase of vial cells to a blue water-nonsoluble formazan crystal product which can be measured spectrophotometrically (Mosmann et al., 1983; Carmichael et al., 1987; Kim et al., 1994b, c, d). Following appropriate incubation of cells (K-562, P-388, FM-3A, U-937 cells) in the presence or absence of synthesized compounds, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, M.O.) was added to each well and incubated at 37°C for a further 4 hours before processing as described below.

For cell growth, serially increasing cell numbers were plated in different columns across 96-well microtiter plates. Well growing cells were harvested, counted and inoculated at the concentrations of 2× 10⁴ cells/ml into 96-well microtiter plates. After 24 hours, synthesized compounds (6a-g and 5a-g) were applied to triplicate culture wells and the culture were incubated at 37°C for 3 days. Following this incubation, 2 μl of MTT solution (5 mg/ml in phosphate buffer solution; KCl 0.2 g, KH_2PO_4 0.2 g, NaCl 8.0 g, Na_2HPO_4 1.15 g, $MgCl_2$ 0.101 g/ml, pH=7.4) was added to microculture wells. After 4 hours incubation at 37°C, the supernatant was removed from each well and 100 μl of 100% DMSO was added to solubilize the formazan crystals which were formed by the celluar reduction of MTT. After thorough mixing with mechanical plate mixer, absorbance spectra was read on ELISA Processor II microplate Reader (Behering Co.) at a wavelength of 570 nm and a reference wavel-

ength of 650 nm (absorbance peak for DMSO). All measurements were carried out in triplicate. There was good reproducibility between replicate wells with standard errors ≤ ±10% (Carmichael, et al., 1987) (Table I).

RESULTS AND DISCUSSION

Chemistry

For the preparation of α-methylene-γ-butyrolactone bearing 6-substituted purines, 5'-methyl-5'-[(9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6a), 5'-methyl-5'-[(6-chloro-9H-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6b), 5'-methyl-5'-[(6-Iodo-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6c), 5'-methyl-5'-[(6-methyl-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6d), 5'-methyl-5'-[(9H-adenin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6e), 5'-methyl-5'-[(6-mercapto-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6f), and 5'-methyl-5'-[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (6g), an efficient Refo-rmatsky type reaction (Ohler et al., 1970) was employed which involves the treatment of ethyl α-(bromomethyl) acrylate (Ferris, 1955) and zinc with the respective ketone intermediates, 1-(6-substituted-9H-purin-9-yl)-2-propanones (5a-g), 1-(9H-purin-9-yl)-2-propanone (5a), 1-(6-chloro-9H-purin-9-yl)-2-propanone (5b), 1-(6-Iodo-9H-purin-9-yl)-2-propanone (5c), 1-(6-methyl-9H-purin-9-yl)-2-propanone (5d), 1-(9H-adenine-9-yl)-2-propanone (5e), 1-(6-mercapto-9H-purin-9-yl)-2-propanone (5f), 1-(9H-hypoxanthin-9-yl)-2-propanone (5g).

The above 6-substituted purine ketone intermediates (5a-g) were directly obtained by the reaction of dry K_2CO_3-treated (or NaH-treated) 6-substituted purine with chloroacetone in the presence of DMF solvent. It has been reported (Montgomery, 1961) that alkylation of 6-chloropurine with iodomethane gave both the N-7 and N-9 position. In this experiment, we found the isomeric mixtures of 1-(6-chloro-9H-purin-9-yl)-2-propanone (5b) and 1-(6-chloro-9H-purin-7-yl)-2-propanone, and 1-(9H-purin-9-yl)-2-propanone (5a) and 1-(9H-purin-7-yl)-2-propanone, with the N-9-substituted product as the predominant. 1-(N6-protected-9H-adenin-9-yl)-2-propanone was directly obtained with the alkylation reaction of chloroacetone and N6-protected adenine. The N6-protection was deprotected with $NaOCH_3$ in CH_3OH to obtain 1-(9H-adenin-9-yl)-2-propanone (5e), which was treated with Reformatsky reaction. 1-(9H-hypoxanthin-9-yl)-2-propanone (5g) was obtained by the reaction of $NaNO_2$ and CH_3COOH with 1-(9H-adenin-9-yl)-2-propanone.

All of compositions and spectral data (IR, ^1H NMR, ^{13}C NMR and MS) were in accord with the assigned structures.
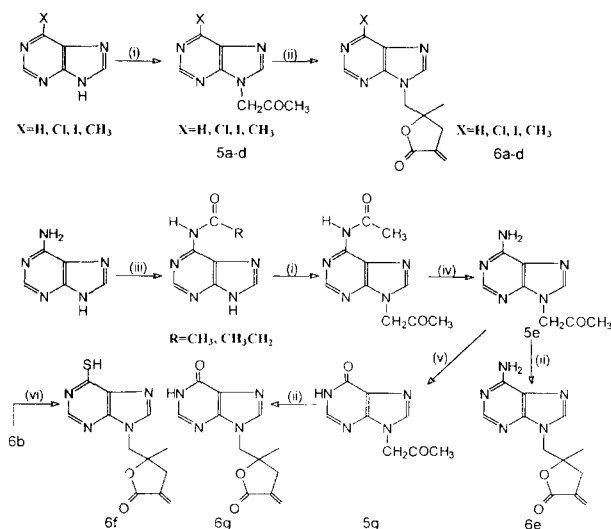
Scheme 1. *Reagant; (i) $ClCH_2COCH_3$, K_2CO_3 (or NaH) DMF (ii) $BrCH_2C$ (=CH_2) COOEt, Zn, N_2, THF, HCl, p-hydroquinone (iii) $(CH_3CO)_2$ or $(CH_3CH_2CO)_2O$, (iv) CH_3ONa, CH_3OH (iv) $NaNO_2/H^+$, (v) Thiourea, 1-propane

Antitumor activity

As illustrated in Table I, the α-methylene-γ-butyrolactone compounds, 5'-methyl-5'-[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylenetetrahydrofuran (**6a-g**) were screened for their in vitro antitumor activity against three cell lines; a) human chromic myelogenous leukemia cell (K-562), b) mouse lymphoid neoplasma cell (P-388) and c) mouse mammary carcinoma cell (FM-3A) and showed the moderate antitumor activity (IC$_{50}$ ranged from 1.4 to 4.3 μg/ml) with the compound, 5'-methyl-5'-[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylenetetra hydr ofuran (**6g**) showing the lest activity. The synthetic intermediate ketone compounds (**5a-g**) were also screened against the above four cell lines and found to be inactive.

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