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Synthesis and Antitumor Evaluation of α-Methylene-γ-butyrolactone-Linked to 5-Substituted Uracil Nucleic Acid Bases

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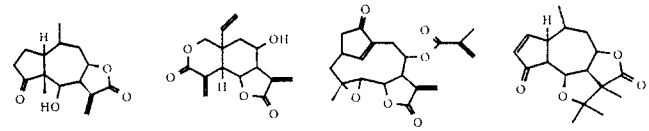
Six, heretofore undescribed, 5'-Methyl-5'-(5-Substituted uracil-1-ylmethyl) -2'-oxo-3'-methylenetetrahydrofurans (F, Cl, Br, I, CH_3, H) (**6a-f**) were synthesized and evaluated against three cell lines (FM-3A, P-388 and U-937). For the preparation of α-methylene-γ-butyrolactone bearing 5-substituted uracils (**6a-f**), the efficient Reformatsky type reaction was employed which involves the treatment of ethyl α-(bromomethyl) acrylate and zinc with the respective 5-substituted uracil-1-ylacetones (**5a-f**). The acetone derivatives (**5a-f**) were directly obtained by the respective alkylation reaction of 5-substituted uracils with chloroacetone in the presence of K_2CO_3 (or NaH). These lactone compounds **6a-f** exhibited moderate to significant activity in all of the three cell lines, and **6b**, **6c** and **6e** showed significant antitumor activities (inhibitory concentrations (IC_{50}) ranged from 1.3-3.8 μg/ml).

Key words : 5'-Methyl-5'-(5-Substituted uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran, α-Methylene-γ-butyrolactone, Cytotoxic moiety, Reformatsky reaction, Antitumor activity, Mouse mammary carcinoma (FM-3A), Mouse lymphoid neoplasma (P-388), Human histiocytic lymphoma (U-937), IC_{50}.

INTRODUCTION

In the search for antitumor agents of plant origin, a number of significantly cytotoxic sesquiterpene lactones have been separated (Kupchan, *et al.*, 1969a,b; Lee, *et al.*, 1972). The biological activity of these complex natural products appears to be associated with their ability to act as alkylating agents by virtue of a Michael-type addition of biological nucleophiles such as L-cysteine, glutathione or thiol-rich enzymes such as phosphofractokinase, glycogen synthetase and DNA polymerase, to the α-methylene-γ-butyrolactone moiety itself (Lee, *et al.* 1976; Kupchan, *et al.*, 1970). This type of system has been observed, for example, in the cytotoxic antitumor agents, helenalin (**1**) (Hall, *et al.*, 1977), vernolephin (**2**) (Kupchan, *et al.*, 1969a, b), elephantophin (**3**) (Kupchan, *et al.*, 1969) and tenulin (**4**) (Hall, *et al.*, 1977). It has been established that the α-methylene-γ-butyrolactone is the most reactive chemical functionality in both **1**, **2**, and **3**, with no reaction being observed between cysteine and the epoxide **3**, or the endocyclic , α,β-unsaturated lactone in **3**. 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 co-workers.



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A large number of possible drug design is to attach an alkylating mustard moiety to a biological carrier. In this way a possible drug candidate bearing the active cytotoxic α-methylene-γ-butyrolactone moiety has been synthesized with a view to developing effective clinical drugs since naturally found derivatives have therapeutic indices that prelude their clinical use (Lee, *et al.*, 1975; Dehal, *et al.*, 1980; Heindel, *et al.*, 1981; Cassady, *et al.*, 1978; Rosowsky, *et al.*, 1974; Sanyal, *et al.*, 1986). 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,

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et al., 1975).

As a part of our effort to develope more useful antitumor agents (Kim, *et al.*, 1992, 1993a,b, 1994a-f, 1995), we were particularly interested in synthesizing suitably substituted nucleic acid bases 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 5-substituted uracil nucleic acid bases **4a-f**, and evaluated these synthesized compounds **6a-f** against three cell lines (FM-3A, P-388 and U-937).

MATERIALS AND METHODS

Melting points were determined on an electrothermal capillary 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 spectra were obtained with a Varian EM-360 spectrophotometer and Varian Gemini 200 MHz (solution in dimethylsulfoxide-d_6 with tetramethylsilane as internal standard). Utraviolet spectra data were measured with a Hitachi 124 spectrometer. The organic solvents and chemicals were obtained from commercial products and purified by the appropriate methods before use. Pertinent data for synthesized compounds (**5a-f, 6a-f**) are listed in Table I and II.

General Procedure for the Preparation of 5-Substituted uracil-1-ylactone (5a-f)

To a stirred solution of 5-substituted uracil (**4a-f**) (3.75 mmol) in DMF (35 ml) was added K_2CO_3 (3.75 mmol). The mixture was continously stirred by adding chloroacetone (3.85 mmol) in small portions during 3-4 hours period. The reaction mixture was evaporated to give oily residues, which were applied to a column packed with silica gel and the colunm was eluted with hexane-ethylacetate (20 : 1, v/v). The fractions containig the **5a-f** were collected first, and concentrated to a syrup which was crystallized from an appropriate solvent (**Table I**).

General Procedure for the Synthesis of Ethyl α-(bromomethyl)acrylate

To a solution of dry, distilled ethylene glycol (25.1 g, 4.05 mol) in dry THF (85 ml) was added cautiously NaH (5.8 g, 0.24 mol). After the first vigorous reaction had subsided, the mixture was heated under reflux for 24 hours. The syrupy suspension of sodium ethylene glycolate resulting was cooled to room temperature and then added slowly to a solution of ethyl β,β'-dibromoisobutyrate (55.6 g, 0.20 mol) in THF (85 ml). The temperatue was kept below 45°C by controlling the rate of reactions when addition was complete, the mixture was stirred for 90 minutes and then poured into water (900 ml). The organic layer which separated was extracted into CH_2Cl_2 (200 ml × 3). The aqueous layer was acidified with aqueous 5 N nitric acid and treated with silver nitrate. After drying, the silver bromide precipitate weighed 20 g (60%). The methylene chloride was evaporated under reduced pressure and the residue was fractionated through the colunm, bp 44-45°C (1.7 mmHg).

Table I. 5-Substituted uracil-1-ylacetone (**5a-f**)

comp.	R	mp(°C)	yield(%)	NMR(DMSO-d6)				UV(H_2O) λ_{max}(nm)	IR(KBr) ϖ(c=o), cm^{-1}	recryt. solvent
				$COCH_3$	N-CH_2	6-H	N-H			
5a	F	223-225	35	2.16	4.58	7.90	11.98	288.1	1730	acetone
5b	Cl	225-227	32	2.16	4.63	7.98	11.92	290.7	1732	acetone
5c	Br	230-231	31	2.16	4.63	8.05	11.53	294.0	1720	acetone
5d	I	234-236	27	2.15	4.62	8.03	11.82	287.9	1719	acetone
5e	CH3	196-198	64	2.15	4.57	7.33	11.23	285.4	1725	$CHCl_3$
5f	H	209-210	65	2.15	4.62	7.45	11.26	279.1	1718	C_2H_5OH

Table II. 5'-Methyl-5'-(5-Substituted uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6a-f**)

comp.	R	mp(°C)	yield(%)	NMR(DMSO-d_6) δ_{ppm}					UV(CH_3Cl) λ_{max}(nm)	IR(KBr) ϖ(c=o),	recryt. slovert
				5'-CH_3	N-CH_2	4'-CH_2	exo-CH_2				
6a	F	224-226	83	1.33	3.98	2.90	5.70	6.18	284.4	1758	$CHCl_3$
6b	Cl	232-233	79	1.33	3.95	2.85	5.72	6.09	286.2	1762	CH_2Cl_2
6c	Br	233-235	78	1.32	3.97	2.87	5.80	6.20	286.0	1766	$CHCl_3$
6d	I	183-185	79	1.32	3.97	2.87	5.79	6.25	298.0	1762	CH_2Cl_2
6e	CH_3	210-212	87	1.35	3.97	2.88	5.70	6.08	282.4	1767	$CHCl_3$
6f	H	200-201	87	1.32	3.95	2.89	5.69	6.15	278.0	1754	$CHCl_3$

General Procedure for the Synthesis of 5'-Methyl-5'-(5-Substituted uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofurans (6a-f)

A solution of ethyl α-(bromomethyl)acrylate (0.67 g, 3.5 mmol) in anhydrous THF (50 ml) was added dropwise with vigorous stirring under nitrogen to a mixture of granulated active Zn (0.33 g, 5.0 mmol), p-hydroquinone (0.004 g) and the 5-substituted uracil-1-ylacetone (**5a-f**) (0.34 g, 2.0 mmol) in anhydrous THF (20 ml). Once the reaction has started, addition was adjusted such that the temperature dose not rise above 40-50°C, The reaction mixture was stirred for about 4 hours at 50°C, cooled and poured into ice-cold 7% HCl (25 ml). The reaction mixture was extracted with chloroform (100 ml×4), and the chloroform extractions were washed with aq $NaHCO_3$, followed by aq NaCl, dried over anhydrous $MgSO_4$. Filtration and evaporation gave residues, which were crystallized from an appropriate solvent (**Table II**)

Evaluation of antitumor activity

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,f).

MTT-Microculture Tetrazolium Assay

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,f). Following appropriate incubation of cells (FM-3A, P-388, and 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 cell were harvested, counted and inoculated at the concentrations of 2×10^4 cells/ml into 96-well microtiter plates. After 24 hours, synthesized compounds (**6a-f** and **5a-f**) were applied to triplicate culture wells and the cultures 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 wavelength of 650 nm (absorbance peak for DMSO). All measurements were carried out in triplicate. There was good reproducibility between replicate wells with standard erors≤+10% (Carmichael, *et al.*, 1987). (**Table III**)

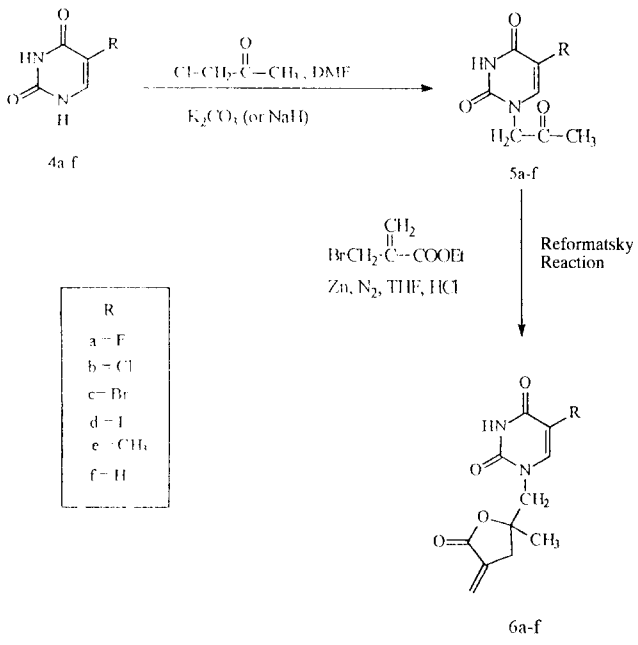
Table III. IC_{50} of 5'-Methyl-5'-(5-Substituted uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofurans and Related Compounds

comp.	IC_{50} (μg/ml)[a]		
	FM3A/S[b]	P388/S[c]	U937/S[d]
5a	24.0	>100	>100
5b	32.0	25	>100
5c	70.0	>100	>100
5d	38.0	>100	>100
5e	42.0	>100	101
5f	18.0	16.5	19.0
6a	5.0	5.3	10.5
6b	1.3	3.3	3.8
6c	3.1	5.8	22.0
6d	2.0	2.8	3.4
6e	1.3	2.5	3.2
6f	1.4	4.3	5.1

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 leukemia cell. d: Human histiocytic lymphoma cell.

RESULTS AND DISCUSSION

For the preparation of α-methylene-γ-butyrolactone-bearing 5-substituted uracils, 5'-methyl-5'-(5-substituted uracil-1-ylmethyl)-2'-oxo-3'-methylene tetrahydrofurans (**6a-f**), the efficient Reformatsky type reaction (Ohler, *et al.*, 1970) was employed which involves the treatment of ethyl α-(bromomethyl) acrylate (Ferris, 1955) and zinc with the respective 5-substituted uracil-1-yl acetone (**5a-f**). The respective yield of the lactones were fairly good. The 5-substituted uracil-1-ylacetones (**5a-f**); 5-fluorouracil-1-ylacetone (**5a**), 5-chlorouracil-1-ylacetone (**5b**), 5-bromouracil-1-ylacetone (**5c**), 5-iodouracil-1-ylacetone (**5d**), thymine-1-ylacetone (**5e**) and uracil-1-ylacetone (**5f**) were directly obtained by the respective reaction of 5-substituted uracil with chloroactone in the presence of K_2CO_3 (or NaH). It has been reported (Montgomery, 1961) uracil was alkylated at the N-1 position. It was also reported that under similar conditions, 5-fluorouracil furnished N-3-substituted derivatives. Recently we have shown (Kim, *et al.*, 1994c,f) that the direct alkylation of 5-fluorouracil with ω-



Scheme

chloroalkyl nitrile afforded moderate yields of the isomeric mixtures of N-1-substituted 1-(ω-cyanoalkyl)-5-fluorouracils and N,N-1,3-disubstituted 1,3-bis(ω-cyanoalkyl)-5-fluorouracils with the N-1-substituted product as the predominant. However, we could not isolate the isomeric 5-fluorouracil-3-ylacetone and analogous 5-substituted uracil-3-ylacetones even after extensive column chromatography and purification. For ^{1}H NMR spectra data of the exocyclic methylene group of all the lactones (**6a-f**), two apparent triplets (J=3-4 Hz) or mutiplets were observed at δ 5.64±0.16 for the proton syn to the lactone carbonyl group and at δ 6.15±0.12 for the proton anti to the carbonyl group. For most of the lactones the C-4 methylene protons of the lactone group were apparent triplet (J= 2-4 Hz) or mutiplets centered at δ 2.85±0.05. The IR spectra of the lactones showed characteristic bands at δ 1754±4 cm^{-1}. The physicochemical data of the lactones and ketones have been described in **Tables I** and **II**.

As illustrated in table III, the 5'-methyl-5'-(5-substituted uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofurans (**6a-f**); 5'-methyl-5'-(5-fluorouracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6a**), 5'-methyl- 5'-(5-chlorouracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6b**), 5'-methyl-5'-(5-bromouracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6c**), 5'-methyl-5'-(5-iodouracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6d**), 5'-methyl-5'-(thymine-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6e**), 5'-methyl-5'-(uracil-1-ylmethyl)-2'-oxo-3'-methylenetetrahydrofuran (**6f**) were evaluated for their in vitro cytotoxicities against three cell lines; a) mouse mammary carcinoma (FM-3A), b) mouse lymphoid neoplasma (P-388), c) human histiocytic lymphoma cell (U-937). The compounds that exhibited moderate to significant activity in all of the three types of cell lines, included **6a-f**, where compounds **6b**, **6c** and **6e** showed significant antitumor activity (inhibitory concentration (IC_{50}) ranged from 1.3 to 3.8 g/ml). The synthetic intermediate compounds 5a-f were also screened against the above three cell lines, and found no activity. Further investigation of the structure-activity relationships of the nucleic acid base α-methylene-γ-butyrolactones and related derivatives is in progress.

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REFERENCES CITED

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., Mitchell, J. B., Evaluation of a Tetrazolium-based Semiautomated Colorimetric Assay; Assessment of Chemosensitivity Testing. *Canceer Res.*, 47, 936-940 (1987).

Cassady, J. M., Bryn, S. R., Stamos, I. K., Evans, S. M., McKenzie, A., Potential Antitumor Agents. Synthesis, Reactivity and Cytotoxicity of α-Methylene Carbonyl Compunds. *J. Med. Chem.*, 21, 815-819 (1978).

Dehal, S.S., Marples, B. A., Stretton, R. J., Traynor, J. R., Steroidal α-Methylenes as Potential Antitumor Agents. *J. Med. Chem.*, 23, 90-92 (1980).

Fursteer, A., Recent Advancements in the Reformatsky Reaction. *Synthesis*, 571-589 (1989).

Ferris, A. F., The Action of Mineral acid on Diethyl bis(hydroxymethyl) malonate. *J. Org. Chem.*, 20, 780-787 (1955).

Gammill, R. B., Wilson, C.A., Bryson, T. A., Synthesis of α-Methylene-γ-butyrolactons. *Synthetic Communication*, 5, 245-268 (1975).

Grieco, P. A., Methods for the Synthesis of α-Methylene Lactones. *Synthesis*, 67-77 (1975).

Hall, I. H., Lee, K-H, Mar, E. C., Starnes, C. O., Waddel, T. G., Antitumor Agents.21. A Proposed Mechanism for Inhibition of Cancer Growth by Tenulin and Helenalin and Related Cyclopentenones. *J. Med. Chem.*, 20, 333-337 (1977).

Heindel, N. D., Minatelli, J. A. Synthesis and Antibacterial and Anticancer Evalutions of α-Methylene-γ-butyrolactones. *J. Pharm. Sci.*, 70, 84-86 (1981).

Kim, J. C., Lee, Y. H. Synthesis and Evaluation of Uracil-6-carboxaldehyde Schiff Base as Potential Antitumor Agents. *Korean J. Med. Chem.*, 2, 64-69

(1992).

Kim, J. C., Park, J. I., Hur, T. H. Synthesis of 4-Aza-cholestane Derivatives Containing Nitrosoureido Function as Antitumor Activity. *Bull. Korean Chem. Soc.*, 14, 176-178 (1993a).

Kim, J. C., Peak, H. D., Moon, S. H., Kim, S. H., Synthesis of Steroidal Cyclophosphamide, 2-bis(2-chloroethyl)amino-2-oxo-6-(5α-cholestanyl)-1,3, 2-oxazaphorinane. *Bull. Korean Chem. Soc.*, 14, 318-319 (1993b).

Kim, J. C. and Han, S. H. Nitrosation Products of N-Acy-N'-Substituted Phenyl Hydazines. *Bull. Korean Chem. Soc.*, 15, 173-176 (1994a).

Kim, J. C., Bae, S. S., Kim, S. H. and Kim, S. H. Synthesis and In Vitro Cytotoxicity of Homologous Series of 9-[ω-(N'-methyl-N'-nitrosoueido) alkyl] purines. Korean *J. Med. Chem.*, 4, 66-72 (1994b).

Kim, J. C., Dong, E. S., Ahn, J. W., Kim, S. H. Synthesis and Evaluation of Antitumor Activity of a Homologous Series of 1-(ω-cyanoalkyl) and 1,3-bis (ω-cyanoalkyl)uracil Nucleoside Analogues. *Arch. Pharm. Res.*, 17, 135-138 (1994c).

Kim, J. C., Dong, E. S., Kim, J. A., Kim, S. H., Park, J. I. and Kim, S. H. Synthesis and Antitumor Evaluation of Acyclic 5-Substituted Pyrimidine Nucleoside Analogues. *Korean J. Med. Chem.*, 4, 111-118 (1994d).

Kim, J. C., Lim, Y. G., Min, B. T. and Park, J. I. Preparation of N'-Substituted Anilino-N-Methyl-N'-Nitrosoureas as Candidate Antitumor Agents. *Arch. Pharm. Res.*, 17, 420-423 (1994e).

Kim, J. C., Dong, E. S., Park, J. I., Bae, S. D. and Kim, S. H. 5-Substituted Pyrimidine Acyclic Nucleoside Analogues. 1-Cyanomethyl- and 1-(4-Cyanobutyl)-5-Substituted Uracils as Candidate Antitumor Agents. *Arch. Pharm. Res.*, 17, 480-482 (1994f).

Kim, J. C., Kim, M. Y., Kim, S. H., Choi, S. H. Synthesis of a Series of cis-Diamminedichloroplatinum (II) Complexes Linked to Uracil and Uridine as Candidate Antitumor Agents. *Arch. Pharm. Res.*, 18, 449-453 (1995).

Kupchan, S. M., Aynehchi, Y., Cassady, J. M., Schones, H. K., Burlingaame, A. L., Tumor Inhibitions XL. The Isolation and Structural Elucidation of Elephantin and Elephantopin, Two Novel Sequiterpenoid Tumor Inhibitors from Elephantopus Elatus. *J. Org. Chem.*, 34, 3867-3875 (1969a).

Kupchan, S. M., Hemingway R. J., Werner, D., Karim, A., Tumor Inhibitors. XLVI. Verlepin, a Novel Sesquiterpene Dilactone Tumor Inhibitor from Vernoniahymenolepis A. Rich. *J. Org. Chem.*, 34, 3903-3908 (1969b).

Kupchan, S. M., Giacobbe, T. J., Krull, I. S., Thomas, A. M., Eakin, M. A., Fessler, D. C. Reaction of Endocyclic α,β-Unsaturated γ-Lactones with Thiols. *J. Org. Chem.*, 35, 3539-3542 (1970).

Lee, K-H., Furukawa, H., Huang, E-S., *J. Med. Chem.*, 15, 6009-611 (1972).

Lee, K-H., Ibuka, T., Kim, S.H., Vestal, B.R., Hall, I.H. Antitumor Agents 16. Steroidal α-Methylene-γ-lactones. *J. Med. Chem.*, 18, 812-817 (1975).

Lee, K-H., Imakura, Y., Sims, D., McPail, A.T., Onan, K.D., *J. Chem. Soc., Commun.*, 341, 1976.

8Mosmann, T., Rapid Colorimetric Assay for Cellular Growth and Survival; Application to Proliferaton and Cytotoxicity Assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Montgomery, J. A., Temple, C., The Alkylation of 5-Chloropurine. *J. Am. Chem. Soc.*, 83, 630-635 (1961).

Ohler, E., Reining, K., Schmidt, U., A Simple Sythesis of α-Methylene-γ-lactones. *Angew. Chem. Internat.* Ed. 9, 457-459 (1970).

Rosowsky, A., Papathanasopoulos, N., Lazarus, H., Foley, G. E., Modest, E. J., *J. Med. Chem.*, 17, 672-676 (1974).

Sanyal, U., Mitra, S., Pal, P., Chakraborti, S. K., New α-Methylene-γ-Lactone Derivatives of Substituted Nucleic Acid Bases as Potential Anticancer Agents. *J. Med. Chem.*, 29, 595-599 (1986).