Potential Antitumor $\alpha$-Methylene-$\gamma$-butyrolactone-Bearing Nucleic Acid Base. 3. Synthesis of 5'-Methyl-5'-[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans

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Search for a new $\alpha$-methylene-$\gamma$-butyrolactone-bearing 6-substituted purine as a potential antitumor agent has led to synthesize seven, hitherto unreported, 5'-Methyl-5'[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (H, CI, I, CH3, NH2, SH, >C=O) (6a-g).

These include 5'-Methyl-5'[(9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6a), 5'-Methyl-5'[(6-chloro-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6b), 5'-Methyl-5'[(6-iodo-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6c), 5'-Methyl-5'[(6-methyl-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6d), 5'-Methyl-5'[(9H-adenin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6e), 5'-Methyl-5'[(6-mercapto-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6f) and 5'-Methyl-5'[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofurans (6g) which were made by the Reformatsky-type reaction of ethyl $\alpha$-(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-(6-mercapto-9H-purin-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 K2CO3 (or NaH) under DMF (or DMSO). The preliminary in vitro cytotoxicity assay for the synthetic $\alpha$-methylene-$\gamma$-butyrolactone compounds (6a-g) were determined against three cell lines (PM-3A, P-388, and K-562) and showed the moderate antitumor activity (IC50 ranged from 1.4 to 4.3 $\mu$g/ml) with the compound 5'-methyl-5'[(9H-hypoxanthin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofuran (6g) showing the least antitumor activity.

Key words: 5'-Methyl-5'[(6-substituted-9H-purin-9-yl)methyl]-2'-oxo-3'-methylene tetrahydrofuran, 6-Substituted-9H-purin-9-yl-2-propanone, Cytotoxic moiety, Antitumor activity, IC50, Reformatsky reaction, Human chronic myelogenous (K-562), Mouse lymphoid neoplasma (P-388), Mouse mammary carcinoma (FM-3A)

INTRODUCTION

The $\alpha$-methylene-$\gamma$-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 $\alpha$-CH2=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 $\alpha$-methylene-$\gamma$-lactone system can act as the alkylation center for cytotoxic antitumor lactones (for example 1, 2, 3 and 4). A Michael-like reaction between biological cellular nucleophiles such as L-cysteine, glutathione or thiol-rich enzymes (phosphofructokinase, glycogen synthase and DNA polymerase), can act to the $\alpha$-methylene-$\gamma$-butyrolactone moiety itself (Lee et al., 1975; Kupchan et al. 1970). It has been established that the
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., 1975; Greeco, 1975; Gammill et al., 1975).

As part of our effort to develop more useful antitumor agents (Kim et al., 1992; 1993a, 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 & Haake Buchler Melting point apparatus and uncorrected. TLC was performed on glass plates coated with silicone oxide (silica gel 60F254) 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 vacuo. 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, 1356, 1205, 1190 cm$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 2.36 (s, 3H), 5.14 (s, 2H), 8.09 (s, 1H), 8.96 (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): 3619, 3060, 2978, 1724, 1607, 1521, 1487, 1421, 1372, 1277, 1178 cm$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 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): 3619, 3060, 2978, 1724, 1607, 1521, 1487, 1421, 1372, 1277, 1178 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): $\delta$ 2.28 (s, 3H), 5.46 (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.

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

<table>
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<th>Comp. NO.</th>
<th>IC$_{50}^a$ (FM-3A)</th>
<th>IC$_{50}^a$ (P-388)</th>
<th>IC$_{50}^a$ (K-562)</th>
<th>Comp. NO.</th>
<th>IC$_{50}^a$ (FM-3A)</th>
<th>IC$_{50}^a$ (P-388)</th>
<th>IC$_{50}^a$ (K-562)</th>
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$^a$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 lymphoid neoplasma.

$^d$Human chronic myelogenous leukemia cell.