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SYNTHESIS AND ANTICANCER EVALUATION OF CERTAIN α-METHYLENE-γ-(4-SUBSTITUTED PHENYL)-γ-BUTYROLACTONE BEARING THYMINE, URACIL, AND 5-BROMOURACIL

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Abstract. Certain α-methylene-γ-(4-substituted phenyl)-γ-butyrolactone bearing thymine, uracil, and 5-bromouracil were synthesized and evaluated for their anticancer activity. These compounds demonstrated a strong growth inhibitory activity against leukemia cell lines. The anticancer potency for the substituents of the lactone C(γ)-phenyl is in an order of 4-Ph > 4-Cl, 4-Br > 4-Me, 4-NO_2 > 4-F. For the pyrimidine portion, 5-bromouracil is more potent than uracil and thymine.

Alkylating agents have played an important role in the development of anticancer drugs. One member of this class of chemotherapeutic agents is α-methylene-γ-butyrolactone bearing helenalin, a natural cytotoxic sesquiterpene.[1,2] The structural requirement for the cytotoxicity is the α-methylene-γ-butyrolactone moiety which acts as an alkylating agent by a *Michael*-type reaction with bionucleophiles.[3] A number of possible drug candidates bearing this functionality had been synthesized with a view of developing effective clinical drugs.[4-6] Although the alkylating anticancer drugs are important and have been clinically used such as cisplatin, chlorambucil, melphalan, and cyclophosphamide. They have a serious drawback which are common to all alkylating agents, they act by alkylating DNA but they have no particular affinity for it. This drawback could in principle be ameliorated by the incorporation of the alkylating pharmacophore onto a DNA-affiniting carrier, which would result in specifically targeting the pharmacophore to the DNA. Thus, synthesis and antitumor evaluation of α-methylene-γ-butyrolactone bearing purine and pyrimidine were explored.[7-10] However, only those α-methylene-γ-butyrolactones with aliphatic methyl and ethyl groups substituted at the lactone C(γ) position were investigated.

As part of our new drug discovery projects, we have synthesized and evaluated certain uracil α-methylene-γ-butyrolactones with methyl, phenyl, and 4-substituted phenyl groups substituted at the lactone C(γ) position.[11,12] The preliminary results indicated that the cytotoxic potency is in an order of 4-substituted phenyl >phenyl >methyl >hydrogen against the growth of KB, Hep-2, HeLa, and Colo-205 cells. In an attempt to better understand the effect of different substituents on C(γ)-phenyl of the lactone with respect to the anticancer activity, the synthesis and extensive evaluation of certain α-methylene-γ-(4-substituted phenyl)-γ-butyrolactone bearing thymine, uracil, and 5-bromouracil were initiated. Their anticancer structure-activity relationships are also discussed.

Synthesis of compounds **8a,b** and **9b** were previously reported.[12] The same synthetic procedures were adopted for the preparation of **7a-f** and **9a** as illustrated in Scheme 1. Alkylation of thymine with 2-bromo-4'-phenylacetophenone under basic conditions provided 1-[2-oxo-2-(4-phenylphenyl)ethyl]thymine (**4a**; 76%

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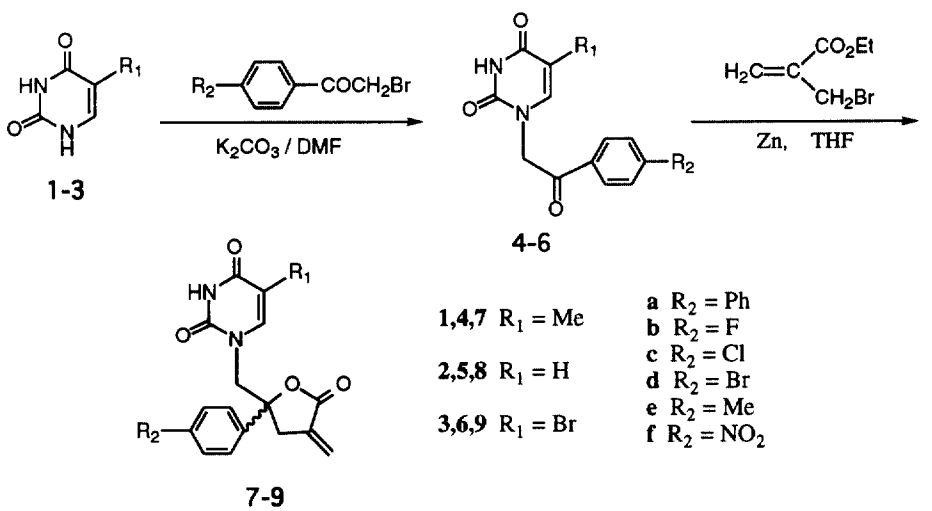
yield) which was then reacted with ethyl 2-(bromomethyl)acrylate and zinc powder in dry tetrahydrofuran (THF) (*Reformatsky*-type condensation) to afford 1-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-(4-phenylphenyl)furan-2-ylmethyl]thymine (**7a**; 93% yield). Accordingly, 1-{[2,3,4,5-tetrahydro-4-methylene-5-oxo-2-(4-substituted phenyl)furan-2-yl]methyl}thymines (**7b-f**; 56-80% overall yield) and 1-{[2,3,4,5-tetrahydro-4-methylene-5-oxo-2-(4-phenylphenyl)furan-2-yl]methyl}-5-bromouracil (**9a**; 63% overall yield) were prepared from 1-[2-oxo-2-(4-substituted phenyl)ethyl]thymines (**4b-f**) and 1-[2-oxo-2-(4-phenylphenyl)ethyl]-5-bromouracil (**6a**) respectively which in turn were obtained *via* alkylation of thymine (**1**), uracil (**2**), and 5-bromouracil (**3**) respectively with aryl bromomethyl ketones.

Scheme 1



All compounds were evaluated *in vitro* against 60 human cancer cell lines derived from nine cancer cell types. For each compound, dose-response curves for each cell line were measured with five different drug concentration, and the concentration causing 50% cell growth inhibition (GI_{50}) compared with the control was calculated. The *in vitro* cancer cells growth inhibitory potency (log GI_{50}) for the substituents of the lactone C(γ)-phenyl is in an order of 4-Ph (**7a**, -5.57) > 4-Cl (**7c**, -5.40), 4-Br (**7d**, -5.39) > 4-Me (**7e**, -5.30), 4-NO_2 (**7f**, -5.26) > 4-F (**7b**, -5.16). Three of these compounds (**7a**, **7c**, and **7d**) exhibited stronger inhibitory activities than that of cisplatin (log GI_{50} = -5.35).[13] A comparable potency of **7e** and **7f** indicated that the inductive effect did not play an important role. The inhibitory activity of **7a,b**, **8a,b**, and **9a,b** against selective cancer cells is outlined in Table 1. These compounds demonstrated a strong growth inhibitory activity against leukemia cell lines but are relatively inactive against non-small cell lung cancers and CNS cancers. A 4-Ph-substituent on the lactone C(γ)-phenyl position **7a** exhibited a better potency than its 4-F-substituted counterpart, **7b**. This is interesting, because a bulky 4-Ph-substituent at the lactone C(γ)-phenyl position usually decreased the cardiovascular activities of α-methylene-γ-butyrolactones.[14-16] Comparison of the mean log GI_{50} values of **8a** (-5.94) and **8b** (-4.99), and that of **9a** (-6.02) and **9b** (-5.22) further confirmed that a bulky 4-Ph group substituted at the lactone C(γ)-phenyl position not only increased the

anticancer potency but also improved the selective cytotoxicity in which the range values (the difference in log GI_{50} values of the least sensitive cancer cell and the most sensitive cancer cell) for **8a**, **8b**, **9a**, and **9b** respectively, are 2.22, 0.83, 2.50, and 1.65. For the pyrimidine portion, 5-bromouracil (**9a,b**) is more potent than uracil (**8a,b**) and thymine (**7a,b**).

Table 1. Inhibition of *in Vitro* Cancer Cell Lines by α-Methylene-γ-butyrolactones [Log GI_{50} (M)][a)]

Cell Line	**7a**	**7b**	**8a**	**8b**	**9a**	**9b**
Leukemia						
HL-60(TB)	-6.63[b)]	-5.59	-6.98[b)]	-5.43	-7.34[b)]	-5.76
RPMI-8226	-6.19	-5.45	-6.67	-5.27	-6.89	-6.33[b)]
Non-Small Cell Lung Cancer						
A549/ATCC	-4.91	-4.75	-4.97	-4.77	-5.21	NT[d)]
NCI-H460	-4.86	-4.72[c)]	-4.99	-4.86	-4.98	-4.68[c)]
Colon Cancer						
COLO 205	-5.85	-5.80[b)]	-6.76	-5.52[b)]	-6.45	-5.65
SW-620	-5.99	-5.44	-6.77	-5.31	-6.55	-5.56
CNS Cancer						
SNB-19	-4.78	-4.83	-4.76[c)]	-4.83	-4.84[c)]	-4.71
U-251	-4.88	-4.94	-5.00	-4.81	-5.24	-5.07
Melanoma						
LOX IMVI	-5.76	-5.37	-6.68	-5.22	-6.07	-5.47
MALME-3M	-5.43	-5.45	-5.80	-5.12	-5.81	-5.34
Ovarian Cancer						
IGROV1	-5.72	-5.26	-6.15	-4.88	-5.92	-4.89
SK-OV-3	-4.87	-4.79	-4.98	-4.82	-5.22	-4.78
Renal Cancer						
ACHN	-5.12	-5.45	-5.64	-4.88	-5.60	-5.35
TK-10	-5.80	-5.34	-6.02	-4.84	-6.90	-4.77
Prostate Cancer						
PC-3	-5.60	-5.04	-5.88	-4.79	-5.90	-4.93
DU-145	-5.78	-5.12	-6.70	-4.82	-5.97	-4.79
Breast Cancer						
HS-578T	-4.59[c)]	-4.72[c)]	-5.13	-4.69[c)]	-5.32	-4.84
MDA-MB-435	-5.70	-5.25	-6.31	-4.97	-6.00	-5.39
Mean[e)]	-5.57	-5.16	-5.94	-4.99	-6.02	-5.22
Range[f)]	2.04	1.08	2.22	0.83	2.50	1.65

a) Data obtained from NCI's *in vitro* disease-oriented cancer cells screen.[17] GI_{50}: Drug molar concentration causing 50% cell growth inhibition.

b) The most sensitive cell.

c) The least sensitive cell.

d) Not tested.

e) Mean values over all cell lines tested. The cell lines used in these experiments were leukemia (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, and SR); non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, and NCI-H522); colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, and SW-620); CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, and U251); melanoma (LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, and UACC-257); ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3); renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, and UO-31); prostate cancer (PC-3 and DU-145); and breast cancer (MCF7, MCF7/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, and T-47D).

f) The difference in log GI_{50} value of the least sensitive cell and the most sensitive cell.

In summary, we have synthesized certain α-methylene-γ-(4-substituted phenyl)-γ-butyrolactone bearing thymine, uracil, and 5-bromouracil. These compounds demonstrated a strong growth inhibitory activity against leukemia cell lines. Among them, 1-{[2,3,4,5-tetrahydro-4-methylene-5-oxo-2-(4-phenylphenyl)furan-2-yl]methyl}-5-bromouracil (**9a**) is the most potent with a mean log GI_{50} value of -6.02 and a range value of 2.50. Synthesis and evaluation of other types of heterocyclic analogues are currently under investigation.

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