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Synthesis and cytotoxic activity of some novel polycyclic γ-butyrolactones

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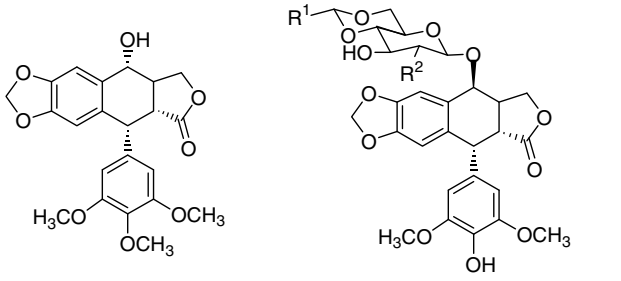
ABSTRACT

Baeyer–Villiger oxidation of 5-aryl-7,11,11-trimethyltricyclo[5.4.0.0^{3,6}]-undec-1-en-4-ones **4a–h** by H_2O_2 and formic acid in methanol yields mixtures of 3b,7,7-trimethyl-3-phenyl-3,3a,3b,4,5,6,7,8a-octahydro-1*H*-indeno-[1,2-c]furan-1-ones **8a–h** and 3b,7,7-trimethyl-3-phenyl-3,3a,3b,4,5,6,7,8a-octahydro-1*H*-indeno-[1,2-c]furan-2-ones **9a–h** in high yields. The obtained butyrolactones **8a–h** display cytotoxic activity against a number of human cancer cells.

Lactone motifs are part of many natural[1–3] and synthetic products,[4–7] which exhibit a variety of biological activities.[4–12] The γ-butyrolactone moiety, that is, the 2(5H)-furanone ring system is part of many oxygenated natural heterocycles[13] and secondary metabolites.[14] The biological activities displayed by such oxygenated heterocyclics include antibiotic, fungicidal, antihelmintic, antitumor, antiviral, anti-inflammatory, cytostatic, antiallergenic, and anti-HIV.[15–22] The known antitumor lactones include podophyllotoxin (**1**), etopside (**2**), and teniposide (**3,** Fig. 1). Though podophyllotoxin (**1**) is an antimitotic agent preventing the polymerization of tubulin,[23] its synthetic anologues (**2**, **3**) inhibit catalytic activity of DNA-topoisomerase II.[24,25] Various approaches, which have been utilized to obtain lactone moiety, include (1) use of catalysts such as CH_3ReO_3/H_2O_2[26] at room temperature in ionic liquids, $RuCl_3$,[27] Fe_2O_3,[28] and bis(dipivaloylmethanato)nickel(II)[29] and (2) use of biocatalyst such as group of Baeyer–Villiger monooxygenase enzymes.[30,31]

Earlier, we had reported[32] that photochemical transformation of (*E*,*E*)-arylidene-β-ionones **4a–e** when irradiated in anhydrous solvent leads to 1,7,7-trimethyl-3-[(*E*)-2′-arylethenyl]-2-oxabicyclo[4.4.0]deca-3,5-dienes **5a–e**, and the latter on UV irradiation in aqueous organic solvent (aq. methanol) are quantitatively transformed to 11-exo-aryl-1,7,7-trimethyltricyclo[4.4.0.1^{2,4}]undec-5-en-3-ones **6a–e**, which rearrange on silica to furnish 5-aryl-7,11,11-trimethyltricyclo[5.4.0.0^{3,6}]undec-1-en-4-ones **7a–e** in high yields. Further, the cyclobutanones **7a–e** were oxidized by air (MeOH solvent) or by formic acid and H_2O_2 to obtain mixture of lactones **8a–e** (Major) and **9a–e** (Minor).[33] Keeping in view the known biological activities of butyrolactones, in particular anticancer activity,[34] it was decided to evaluate the cytotoxic activity of lactones against human cancer cell lines. For this purpose, in addition to the earlier reported (*E*,*E*)-arylidene-β-ionones **4a–3e**, new (*E*,*E*)-arylidene-β-ionones bearing electron-withdrawing group on the aryl ring (*p*-CN,*p*-F), **4f**, **g** and 2-furfuralyidene-β-ionones, **4h**, were also synthesized by the earlier reported procedure.[32,33]

The (*E*,*E*)-arylidene-β-ionones **4a–h** were resynthesized and irradiated under similar conditions as reported earlier[32,33] to ob-



1 Podophyllotoxin

2 Etoposide R^1= CH3; R^2 = H

3 Teniposide R^1= C_4H_3S; R^2 = H

Figure 1.

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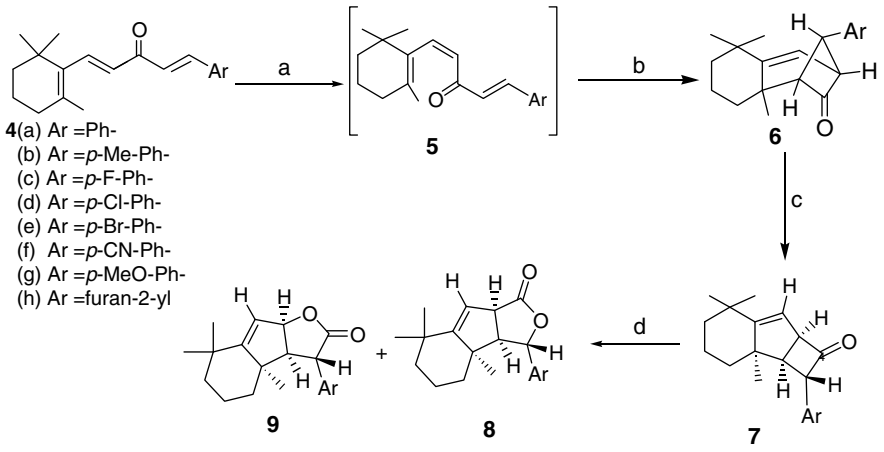

Scheme 1. Synthesis of some new γ-butyrolactones derivatives. Reagents and conditions: (a) aqueous MeOH or anhydrous benzene, hν; (b) aqueous MeoH, hν; (c) rearrangement on silica 60–120 mesh; (d) formic acid and H_2O_2 in MeOH.

Table 1
Reaction yield (%) of the products **8** and **9**

Arylidene-β-ionones	Reaction time (h)	Yield of **8** (%)	Yield of **9** (%)
4a	**3**	70	13
4b	**3**	70	15
4c	**3**	73	13
4d	**4**	70	12
4e	**4**	74	15
4f	**3**	72	16
4g	**4**	73	13
4h	**4**	75	14

tain 5-aryl-7,11,11-trimethyltricyclo[5.4.0.0^{3,6}]undec-1-en-4-ones **7a**–**h**, the latter were oxidized in formic acid and H_2O_2 (BV oxidation) at room temperature, in methanol, to obtain these γ-butyrolactones **8a**–**e** and **9a**–**e** in good yields; these were separated by column chromatography (Scheme 1, Table 1) using silica 60-120 mesh (Loba Cheme, 20 g, packed in hexane) and eluted with 5% EtOAc in hexane. All the intermediates and the final products have been characterized by detailed spectroscopic (^{1}H and ^{13}C NMR, IR and Mass) and microanalytical data;[35] X-ray crystallographic structure for **8c** was reported earlier (CCDC-169322).[33]

In vitro cytotoxic studies of lactones **8a**–**h** were carried out on different cancer cell lines according to the protocol of Skehan et al.[36,37] The cytotoxic effects of γ-butyrolactones were observed on lungs (A-549 and HOP-62), colon (HCT-15 and COLO-205), ovary (IGR-OV-1 and SK-OV-3), prostrate (DU-145), liver (HEP-2), and human central nervous system (SF-295) cancer cell lines. IC_{50} values (μM), which is the concentration required to inhibit

Table 2
In vitro cytotoxicity of γ-butyrolactones **8a**–**h** against human cancer cell lines

Compounds/standard drugs	Conc. (μM)	% Growth inhibition against human cancer cell lines[a]								
		A-549 Lung	HOP-62 Lung	HCT-15 Colon	COLO-205 Colon	IGR-OV-1 Ovary	SK-OV-3 Ovary	DU-145 Prostrate	HEP-2 Liver	SF-295 CNS
8a	10	11	16	9	14	0	20	15	6	15
	50	29	21	46	34	22	30	32	18	18
	100	**51**	28	**56**	**79**	**63**	**57**	**56**	37	22
8b	10	13	2	11	10	0	19	6	9	19
	50	29	13	44	22	29	29	10	25	20
	100	42	21	**55**	34	40	30	35	27	21
8c	10	2	9	13	11	7	6	0	7	4
	50	45	14	34	26	20	13	0	13	10
	100	**51**	27	38	32	29	41	35	23	15
8d	10	10	27	9	22	1	10	16	8	20
	50	12	29	29	24	15	14	30	18	23
	100	20	38	30	25	16	24	35	18	31
8e	10	8	0	7	14	7	6	21	3	0
	50	10	**55**	32	14	26	28	27	18	18
	100	26	**66**	40	32	30	**52**	28	22	21
8f	10	15	5	14	2	6	17	16	5	12
	50	24	9	25	24	25	25	22	18	17
	100	25	18	30	24	29	27	25	19	20
8g	10	8	10	16	0	3	0	8	3	0
	50	31	30	45	15	30	0	12	23	16
	100	**55**	**52**	**55**	**52**	**51**	21	43	27	16
8h	10	19	2	14	16	1	6	17	8	0
	50	20	8	**50**	20	24	9	37	19	17
	100	25	46	**56**	**64**	**67**	**57**	**76**	**52**	38
Paclitaxel	10	40	26	40	9	15	—	52	29	22
5-Fluorouracil	20	57	37	30	27	29	46	29	77	11
Adriamycin	10	47	69	54	—	—	—	64	—	54
Mitomycin-C	10	55	—	—	—	—	—	63	—	—

[a] % age growth inhibition, % age inhibition caused by the compounds and standard drugs at various concentrations.

Table 3
IC_{50} value for different cancer cell lines

Compound	IC_{50}[a] (μM)								
	A-549 Lung	HOP-62 Lung	HCT-15 Colon	COLO-205 Colon	IGR-OV-1 Ovary	SK-OV-3 Ovary	DU-145 Prostrate	HEP-2 Liver	SF-295 CNS
8a	**97.6**	>100	**75.28**	**63.6**	**83.7**	**86.5**	**87.61**	>100	>100
8b	>100	>100	**78.04**	>100	>100	>100	>100	>100	>100
8c	**79.34**	>100	>100	>100	>100	>100	>100	>100	>100
8d	>100	>100	>100	>100	>100	>100	>100	>100	>100
8e	>100	**49.5**	>100	>100	>100	**95.1**	>100	>100	>100
8f	>100	>100	>100	>100	>100	>100	>100	>100	>100
8g	**89.1**	**94.8**	**77.13**	**99.2**	**94.2**	>100	>100	>100	>100
8h	>100	>100	**50.0**	**79.5**	**79.2**	**91.0**	**63.28**	**98.8**	>100

[a] IC_{50}, 50% inhibitory concentration represents the mean from dose response curves of number of experiments.

cancer cell proliferation by 50% after exposure of cells to test compounds, have also been determined (Table 3). In the case of the lung cancer cell line HOP-62, the maximum inhibition of 66% at 100 μM was observed for **8e** with IC_{50} = 49.5. For cell line A-549, the maximum inhibition was 55% (100 μM) for **8g** with IC_{50} = 89.1. In the case of colon (COLO-205), the maximum inhibition was 79% (100 μM) for **8a** with IC_{50} = 63.6, followed by **8h**, 64%, at same concentration, with IC_{50} = 79.5. In the case of colon cell line HCT-15, the maximum inhibition of 56% (100 μM) was observed for **8a** and **8h** with IC_{50} = 75.28 and 50.0, respectively, which is followed by 55% at same concentration for **8b** and **8g** with IC_{50} = 78.04 and 77.13, respectively. The ovarian cancer cell line IGR-OV-1 shows maximum inhibition of 67% (100 μM) with **8h** having IC_{50} = 79.2, followed by 63% at the same concentration for **8a** having IC_{50} = 83.7. In a ovarian cancer cell line SK-OV-3, the inhibitory potential of 57% (100 μM) was observed for **8a** and **8h** with IC_{50} = 86.5 and 91.0, respectively. In the case of prostrate cancer cell line DU-145, the maximum inhibition of 76% at 100 μM was shown by **8h** with IC_{50} = 63.28. In the case of liver cell line HEP-2 the inhibition observed was 52% (IC_{50} = 98.8) for **8h**. In prostrate cell line DU-145 the maximum inhibition is 76% was observed **8h** (IC_{50} = 63.28). The inhibitory effect on CNS was also evaluated using SF-295 cell line (Table 2). The results indicate that some of the lactones such as **8a**, **8g**, and **8h** are active against a number of human cancer cells. Though mode of action is yet to be established, γ-butyrolactones such as podophylotoxin are tubulin polymerase inhibitor,[23] and their synthetic analogues such as etoposide are topoisomerase-II inhibitor.[24,25] On the other hand, α-alkylidene γ-butyrolactones can act as Michael acceptors in reactions with bionucleophiles, especially mercapto groups present in the cystein residue and their cytotoxic potential related to reactivity toward biological nucleophile.[22]

In conclusion, lactones **8a**–**h** were synthesized and evaluated for anticancer activity against human cancer cell lines. The results of investigations indicate that in most of the experimental observations, maximum activity was observed when aromatic ring is either unsubstituted or bears electron-releasing groups, that is, Me, MeO, and Furan-2yl. Compounds **8a**, **c**, **e**, **g** are active on lung, **8a**, **e**, **g**, **h** on ovary, **8h** on liver, and **8a**, **h** on prostrate cancer, whereas no significant inhibition was observed on CNS cancer cells. These lead lactones may be developed further.

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3-(4-Fluorophenyl)-3b,7,7-trimethyl-3,3a,3b,4,5,6,7,8a-octahydro-1H-indeno[*1,2-c*]*furan-1-one* (**8g**): Colorless solid, mp 113–115 °C (hexane). ν_{max} (KBr): 1757(C=O), 1731, 1610, 1517, 1458, 1373, 1355, 1334, 1309, 1265 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.14(s, CH_3), 1.17(s, CH_3), 1.22(s, CH_3), 1.37–1.58(m, 6H, 3× CH_2), 2.81(t, *J* = 7.8 Hz, 1H, C3aH), 3.76(dd, *J* = 7.0 and 1.8 Hz, 1H, C8aH), 5.28(d, *J* = 8.4 Hz, 1 H, C3H), 5.35(d, *J* = 1.5 Hz, 1H, C8H), 6.99–7.08 (m, 2H, ArH), 7.23–7.37 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.30(C5), 27.78(CH_3), 28.45(CH_3), 30.98(CH_3), 33.88(C4) 34.17(C3b), 40.16(C6), 49.52(C7), 50.67(C3a), 59.38(C8a), 81.45(C3), 115.53(q), 115.61(C8), 128.04(CH), 134.83(q), 160.10(C7a), 162.52(CH), 176.16(C=O). MS (ESI): *m*/*z* 337($M+Na^+$); Anal. calcd. For $C_{20}H_{23}FO_2$: C, 76.41; H, 7.37. Found: C, 76.38; H, 7.34.
3-(Furan-2-yl)-3b,7,7-trimethyl-3,3a,3b,4,5,6,8a-octahydro-2-oxa-cyclopenta[*a*]*inden-1-one* (**8h**) yellowish oil (Hexane), ν_{max} ($CHCl_3$): 1766 (C=O), 1612, 1581, 1458, 1303, 1272, 1249 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.14 (s, CH_3), 1.15 (s, CH_3), 1.21(s, CH_3), 1.37–1.54 (m, 6H, 3× CH_2), 3.03 (t, *J* = 7.6 Hz, 1H, C3aH), 3.73 (dd, *J* = 6.9 and 1.8 Hz, 1H, C8aH), 5.27 (d, *J* = 8.7 Hz, 1H, C3H), 5.33 (d, *J* = 1.8 Hz, 1H, C8H), 6.06(d, *J* = 3.3 Hz, 1H, C2′H), 6.38 (dd, *J* = 1.8 and 3.3 Hz, 1H, C3′H), 7.41(d, *J* = 1.8, 1H, C4′H); MS (ESI): *m*/**z** 309 ($M+Na^+$); Anal. calcd For $C_{18}H_{22}O_3$: C, 76.50; H, 7.74. Found: C, 76.45; H, 7.68.
36. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMohan, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107. For evaluating cytotoxicity of compounds **8a**–**h**, which were dissolved in DMSO, a stock solution of 2×10^4 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/ml gentamycin to obtain test concentrations of 100, 50, 10 μM. Adriamycin and paclitaxel were dissolved in DMSO, and stock solution of 2×10^3 μM was prepared. 5-Fluorouracil and Mitomycin-C were dissolved in double distilled water, and stock solution of 2×10^3 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/ml gentamycin to obtain desired concentrations (Table 2). All the cells were maintained in RPMI-1640 medium, supplemented with fetal bovine serum (10%), 100 U/ml penicillin and 100 μg/ml streptomycin (complete medium). The cells were seeded into 96-well cell culture plates (1×10^4 cells/100 μl/well) and incubated in CO_2 incubator (37 °C, 5% CO_2, 95% relative humidity) for 24 h. After 24 h, compounds **8a**–**h** and positive controls (100 μl/well) were added in quadruplets, and the plates were further incubated in CO_2 incubator for 48 h. Suitable controls were also included in each experiment. After 48 h, chilled trichloro acetic acid (50% w/v, 50 μl) was laid gently on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells. All the contents of the wells were gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloro acetic acid, growth medium, low molecular weight metabolites and serum proteins, etc. The plates were air-dried. Sulforhodamine-B (0.4% SRB in 1% acetic acid, 100 μl/well) was added to each well of the 96-well plates for 30 min. Excess of the dye was washed off using 1% acetic acid, and the plates were air-dried. Tris buffer (10 mM, pH 10.5, 100 μl/well) was added to each well, and plates were shaken on a mechanical stirrer for 10 min, and O.D. was recorded on ELISA reader at 540 nm. Viability of cells was evaluated by trypan blue exclusion method immediately before setting up the experiment for cytotoxicity determination. Cells with >98% viability were used in the assay.[38].
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