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Original article

Isochaihulactone analogues: Synthesis and anti-proliferative activity of novel dibenzylbutyrolactones

Babak Heidary Alizadeh [a], Alireza Foroumadi [b,c], Saeed Emami [d], Mehdi Khoobi [c], Fatemeh Panah [e], Sussan K. Ardestani [e], Abbas Shafiee [b,*]

[a] Iranian Research Institute of Plant Protection (IRIPP), Shahid Chamran Avenue, Yaman St. No 1; PO.Box:1454, Tehran, Iran
[b] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
[c] Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
[d] Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[e] Institute of Biochemistry and Biophysics, Department of Biochemistry, University of Tehran, PO Box 13145-1384, Tehran, Iran

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ABSTRACT

A series of dibenzyl-γ-butyrolactones bearing a hydroxyl group at the benzylic position of 3-benzyl group were synthesized as hydrated analogue of isochaihulactone and evaluated against breast cancer human cell lines (MDA-M231, MCF-7 and T47D). The target compounds were synthesized in 7 steps from known lactone; (*S*)-(+)-γ-benzyloxymethyl-γ-butyrolactone. The key step was the aldol condensation between (+)-(*R*)-β-(benzo[*d*][1,3]dioxol-5-ylmethyl)-γ-butyrolactone and substituted benzaldehydes which afforded corresponding α-hydroxybenzyl butyrolactone analogues. The cytotoxic study of the synthesized compounds against breast cancer human cell lines showed that some of them inhibit breast cancer human cell proliferation with percentage inhibitions over 50% at concentrations less than 50 μg/mL.

1. Introduction

Cancer is a serious clinical problem and poses significant socio-economical effects on the human healthcare. Despite improved imaging and molecular diagnostic techniques, the disease still impacts millions of patients worldwide [1]. Chemotherapy has still been an important treatment modality for cancers. However, toxicity and poor tolerance to current chemotherapeutic agents are dose-limiting factors. This has led to a rising interest in developing new anticancer drugs [2].

Dibenzylbutyrolactone framework is part of many natural and synthetic compounds, which exhibit a variety of biological activities including antibiotic, fungicidal, antiviral, anti-HIV, anti-helmintic, anti-inflammatory, antiallergenic and antitumor [3–8]. The known antitumor lactones including podophyllotoxin (**1**), etopside (**2**), and teniposide (**3**, Fig. 1) also can be considered as rigid dibenzylbutyrolactones [9,10]. Isochaihulactone **4** is one of the famous lignans in α-bezylidene-β-methylenedioxybenzyl-γ-lactones family that isolated from an important Chinese herb Nan-Chai-Hu (Chai Hu of the South), the root of *Bupleurum scorzonerifolium* [1]. This compound exhibited cytotoxic activity in a variety of human tumor cell lines and also inhibited tubulin polymerization in a concentration-dependent manner. The butyrolactone moiety, that is, the 2(5*H*)-furanone ring system plays a role as a pharmacophore for antitumor activity. Among dibenzylbutyrolactones, only a few *trans*-α,β-dibenzyl-γ-butyrolactones, carrying a hydroxyl group at the benzylic position of β-benzyl group are known in nature that exemplified by (−)-para-benzlactone **5**. It was found that the presence of oxygen at the benzylic position affected the antioxidant and cytotoxic activity of dibenzylbutyrolactone and dibenzyltetrahydrofuran lignans [11,12].

Following our studies on the synthesis and cytotoxic activities of non-natural compounds [13–16], in this paper we have synthesized and evaluated a series of dibenzyl-γ-butyrolactones **6a**–**f**, bearing a hydroxyl group at the benzylic position of α-benzyl group instead of β-benzyl group (Fig. 1). Also, compound **6g** was synthesized as a des-hydroxy analogue. The methylenedioxybenzyl group, which is one of the main benzyl groups of cytotoxic butyrolactones was kept as a benzyl structure at the 4-position of 2(5*H*)-furanone system.

* Corresponding author. Tel.: +98 21 66406757; fax: +98 21 66461178.
E-mail address: ashafiee@ams.ac.ir (A. Shafiee).

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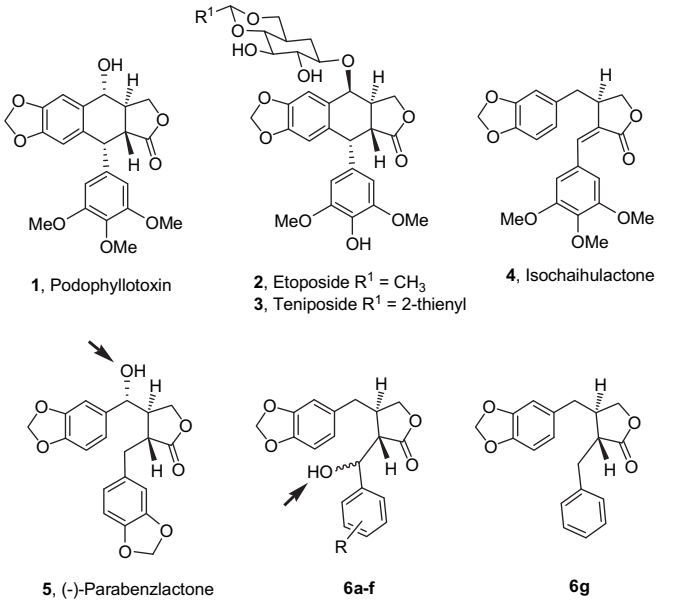
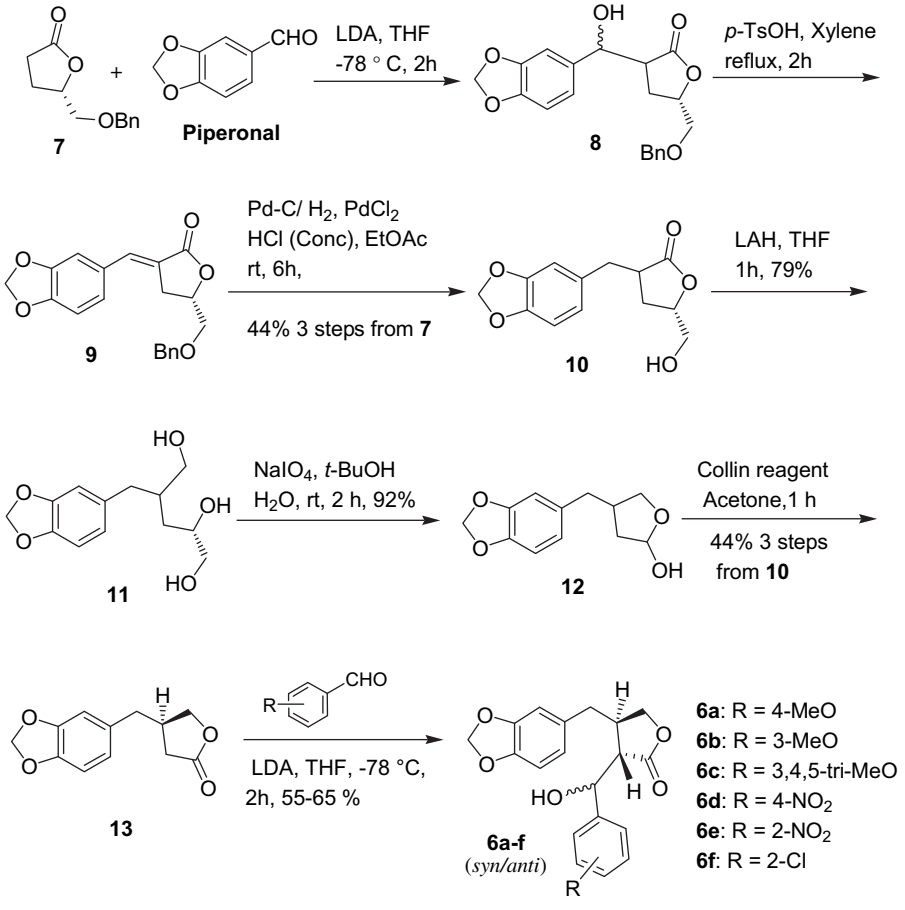


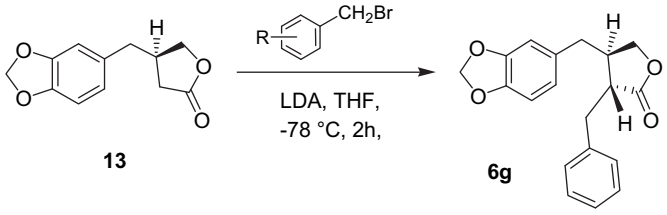
Fig. 1. Structures of well-known γ-butyrolactones **1**–**5** and designed dibenzyl-γ-butyrolactones **6a**–**g**.

2. Chemistry

As shown in Scheme 1, the desired compounds **6a**-**f** were prepared according to known procedure [17,18]. The synthetic rout was started from the reaction of (*S*)-(+)-γ-benzyloxymethyl-γ-butyrolactone (**7**) [19] and piperonal in the presence of LDA at low temperature. The crude product **8** was dehydrated in refluxing xylene and purified by chromatography to afford benzylidenebutyrolactone **9**. The crude product **9** was converted to alcohol **10** using Pd-C/H_2 hydrogenation (44% yield for three steps). The hydroxymethyllactone **10** was treated with LAH to give opening-ring triol **11**, which was cyclized to lactol **12** using sodium periodate in high yield. The Collin's oxidation of lactol **12** afforded the key intermediate benzyl butyrolactone **13** (55% yield for 3 steps). As can be seen in Scheme 1, treatment of lactone **13** with LDA at − 78 °C in the presence of HMPA in THF, deprotonated the α-position of carbonyl group, subsequent treatment of this anion with substituted benzaldehydes yielded dibenzyl-γ-butyrolactones **6a**-**f** bearing hydroxyl group at benzylic position. For preparation of analogue **6g** (without hydroxyl group at benzylic position), the lactone **13** was treated with benzyl bromide instead of benzaldehyde derivatives (Scheme 2). The aldol products **6a**–**f** were mixture of two *syn*/*anti* diasteromers based on the *S*- or *R*-configuration of C_7 benzylic hydroxyl group (Fig. 2). The ^{1}H-NMR showed two sets of signals corresponding to structures *syn* and *anti*. The relative configuration at C_7 was established based on the chemical shifts of H_7 (downfield shifted by the carbonyl group in the *syn*-isomer) and H_7–H_3 coupling constant (larger in *anti*-isomer). Due to the formation of a hydrogen bond between hydrogen of the C_7 benzylic hydroxyl group and oxygen of the carbonyl group, the H_7-H_3 relationship of the *syn*-isomer is axial-equatorial and that of the *anti*-isomer is di-axial. Thus, the observed coupling constant between H_7 and H_3 of *syn*-isomer was lower



Scheme 1. Synthesis of compounds **6a**–**f**.



Scheme 2. Synthesis of compounds **6g**.

than that of *anti*-isomer in accordance with the data given in the literature [20]. In the case of **6g**, only one enantiomer was obtained with $[\alpha]_D^{25} + 4.8^\circ$ ($c = 0.0016$, CH_2Cl_2).

3. Pharmacology

The target compounds were subjected to in vitro anticancer assay against breast cancer human cell lines including MDA-MB 231, MCF-7 and T47D (human breast duct carcinoma; ATCC HTB-133 estrogen receptor-positive, ER^+). Cell survival was determined by MTT colorimetric assay. In this assay, the viable cell number is directly proportional to the production of formazan, which, following solubilization with isopropyl alcohol, can be measured spectrophotometrically at 492 nm by an ELISA plate reader. The compounds were tested at concentrations of 25 and 50 μg/mL for MDA-MB 231 and MCF-7 cell lines and at the dose of 25 μg/mL for T47D cells. The percentages of growth inhibitions over the tested cell lines were determined (Table 1). Doxorubicin and tamoxifen were used as standard drugs.

4. Results and discussion

As shown in Table 1, most compounds reduced tumor cells viability at the doses of 25 or 50 μg/mL after 24 h incubation. Based on these data, dose of 25 μg/mL of compound *syn*-**6f** reduced MDA-MB 231 cell viability by 34.8% while other compounds showed less inhibitory activity at this concentration. The growth inhibitory activities of standard drugs doxorubicin and tamoxifen against MDA-MB 231 cells at the concentration of 25 μg/mL were about 58%. Compounds carrying mono-substitution on benzyl ring showed percentage inhibition of MDA-MB 231 cell proliferation ranging from 20% to 48.8% at 50 μg/mL concentration and compounds **6a** (4-methoxy analogue) and *syn*-**6f** (2-chloro derivative) exhibited more potent inhibitory activity. A significant difference in MDA-MB 231 cell growth inhibition was observed between *syn* and *anti*-isomers of compound **6f**. Indeed, *syn*-**6f** showed more inhibitory activity against MDA-MB 231 cells than *anti*-**6f**. Similarly, by comparing the inhibitory activity of the *syn*- and *anti*-isomers of compound **6d** at different concentrations against MDA-MB 231 cell line, it is observed that *syn*-**6d** inhibited cell growth more effectively than *anti*-**6d**. The introduction of polymethoxy on benzyl ring (compound **6c**) could not lead to an improvement in the anti-proliferative activity against MDA-MB 231 cells in comparison with mono-methoxy compounds (**6a** and **6b**).

In the case of MCF-7 cells, des-hydroxy analogue (compound **6g**) showed the most growth inhibitory activity, reducing cell viability by 46% at the dose of 50 μg/mL, suggesting that a hydroxy group at benzylic position is not important for cell growth inhibition. Moreover, the percentage of inhibition against MCF-7 cells was more than 20% for compounds **6a**, **6c**, **6d** and *anti*-**6f** at this concentration. The highest inhibition (65.7%) against MCF-7 cell line was exhibited by tamoxifen at the concentration of 25 μg/mL.

Compounds **6c**, **6e**, *syn*-**6f** and **6g** were also tested against T47D cell line at the dose of 25 μg/mL (Table 1). These compounds exhibited ≥ 50% inhibition against T47D cells, with the exception of **6e** which showed only ~38% inhibition at this concentration.

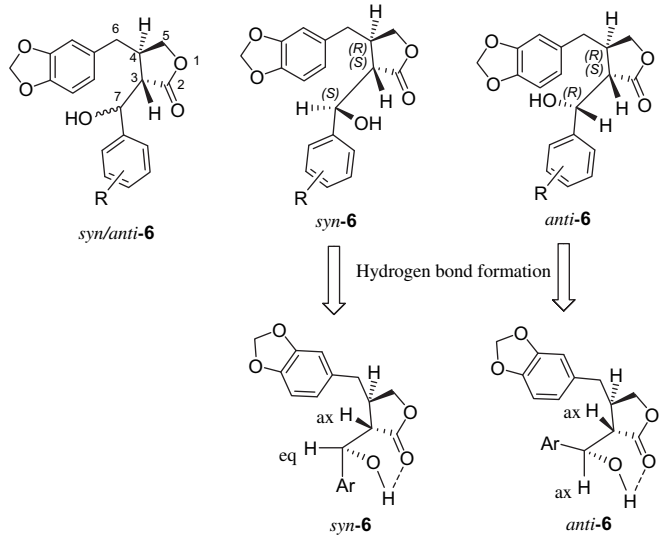


Fig. 2. The atom numbering of compounds **6a**–**f** (used only for NMR data); and formation of a hydrogen bond between C_7–OH and oxygen of the carbonyl group in *syn* and *anti* structures.

5. Conclusion

In conclusion, a series of dibenzyl-γ-butyrolactones bearing a hydroxyl group at the benzylic position of α-benzyl group were synthesized as hydrated analogue of isochaihulactone and evaluated against breast cancer human cell lines (MDA-M231, MCF-7 and T47D). Although none of the newly synthesized compounds showed more activity than standard drugs (doxorubicin and tamoxifen), some of them inhibited breast cancer human cell proliferation at 50 μg/mL concentration with percentage inhibitions over 50% and can be considered for further structural optimization and development as potential anticancer agents for the treatment of breast cancer.

6. Experimental

6.1. Chemistry

All chemicals and reagents were obtained from Merck and Sigma–Aldrich Chemical Companies. ^{1}H-NMR spectra were measured using a Bruker 80 and 500 spectrometers (Brucker, Rheinstetten, Germany) and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrographs (KBr disks) (Nicolet, Madison, WI, USA). MS spectra were obtained with a Finnigan MAT TSQ-70 spectrometer (Finnigan Mat, Bremen, Germany). The purity of all compounds was confirmed by thin-layer chromatography (TLC) using different mobile phases. The results of elemental analyses (C, H, N) were within ± 0.4% of theoretical values for C, H, and N.

Compound **8**–**13** were prepared by known procedures [18,19].

6.1.1. (5S)-3-(Benzo[d][1,3]dioxol-5-yl(hydroxy)methyl)-5-(benzyloxymethyl)- dihydrofuran − 2(3H)-one (**8**)

^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.85 (m, 2H, CH_2), 3.16 (m, 1H, -CH-CO), 3.58 (m, 2H, -CH_2-O), 4.5 (s, 2H, -O-CH_2-Ph), 4.71 (m, 1H, -CH-O), 5.23 (br s, 1H, CH-Ar), 5.93 (s, 2H, -O-CH_2-O), 6.77 (m, 3H, aromatic-H), 7.31 (m, 5H, Ph).

Table 1
The percentages of growth inhibition of the compounds **6a**–**g** against the selected tumor cell lines at the concentrations of 25 and 50 μg/mL.

Compound[a]	R	MDA-MB 231[b]		MCF-7		T47D
		25 μg/ml	50 μg/ml	25 μg/ml	50 μg/ml	25 μg/ml
6a	4-OMe	10.1 ± 2.2	48.8 ± 4.2	42.7 ± 9.3	22.4 ± 2.7	nt[c]
6b	3-OMe	14.5 ± 3.6	41.9 ± 5.5	−3.8 ± 1.6	12.2 ± 2.2	nt
6c	3,4,5-$(MeO)_3$	−1.9 ± 1.1	10.2 ± 7.6	5.2 ± 2.2	37.7 ± 3.9	50.1 ± 17.5
6d[d]	4-NO_2	22.5 ± 3.5	29.0 ± 1.05	7.2 ± 4.8	38.8 ± 16.1	nt
***syn*-6d**	4-NO_2	17.9 ± 5.1	20.1 ± 5.3	24.6 ± 5.3	17.9 ± 8.3	nt
6e	2-NO_2	7.9 ± 1.9	35.1 ± 13.6	21.9 ± 8.6	−0.2 ± 4.4	38.1 ± 25.3
***syn*-6f**	2-Cl	34.8 ± 8.9	47.3 ± 6.8	15.8 ± 6.3	7.5 ± 2.6	50.2 ± 17.1
***anti*-6f**	2-Cl	19.0 ± 13.3	26.4 ± 3.7	8.5 ± 2.3	31.9 ± 11.2	52.9 ± 24.2
6g		26.2 ± 3.0	36.2 ± 3.3	17.0 ± 12.4	46.0 ± 14.0	61.9 ± 12.0
Doxorubicin		57.9 ± 7.9	48.8 ± 6.2	54.6 ± 11.8	31.8 ± 12.0	60.0 ± 3.3
Tamoxifen		58.6 ± 4.4	67.7 ± 4.4	65.7 ± 15.7	25.0 ± 7.5	67.8 ± 5.9

[a] Compounds **6a**–**c** and **6e** were tested as a mixture of *syn* and *anti*-isomers.
[b] Percent inhibition of cell proliferation at different concentrations; mean ± SD values of three independent experiments are reported.
[c] not tested.
[d] *anti*-isomer + trace of *syn*.

*6.1.2. (5S)-3-(Benzo[d][1,3]dioxol-5-ylmethylene)-5-(benzyloxymethyl)-dihydrofuran- 2(3H)-one (**9**)*

^{1}H-NMR (80 MHz, $CDCl_3$) δ: 3.11 (m, 2H, -CH-CH_2-CH), 3.69 (d, 2H, *J* = 4.8 Hz, -O-CH_2-CH), 4.58 (s, 2H, -O-CH_2-Ph), 4.7 (m, 1H, -CHO-), 6.0 (s, 2H, -O-CH_2-O), 6.90–7.02 (m, 3H, aromatic-H), 7.30 (m, 5H, Ph), 7.46 (t, 1H, *J* = 3 Hz, -CH=C).

*6.1.3. (5S)-3-(Benzo[d][1,3]dioxol-5-ylmethyl)-5-(hydroxymethyl)-dihydrofuran-2(3H)-one (**10**)*

Yield 44% (3 steps from **7**); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.9–4.3 (m, 5H, CH_2–CH–CH_2-), 4.46 (m, 1H, -CH-O), 5.89 (m, 2H, -O-CH_2-O), 6.68 (m, 3H, aromatic-H).

*6.1.4. 4-(Benzo[d][1,3]dioxol-5-ylmethyl)-tetrahydrofuran-2-ol (**12**)*

^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.78 (m, 2H, -CH-$\underline{CH_2}$-CH), 1.8–2.3 (m, 1H, Ar–CH_2–CH), 2.6–2.8 (m, 2H, Ar-CH_2), 2.85 and 3.00 (two brs, total 1H, OH), 3.4–4.6 (m, 2H, -CH-CH_2-O), 5.5 (m, 1H, -CH-(OH)-O), 5.94 (s, 2H, -O-CH_2-O), 6.4–6.8 (m, 3H, aromatic-H).

*6.1.5. (+)-(R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-dihydrofuran-2 (3H)-one (**13**) [18]*

$[\alpha]_D^{25}$ + 46.8° (*c* = 0.0016, CH_2Cl_2) as a pale yellow oil (55% for 3 steps from **10**); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.29 (dd, 1H, *J* = 17.5, *J* = 7.1 Hz, -H_3), 2.60 (dd, 1H, *J* = 17.5, *J* = 8.2 Hz, -H_3), 2.67 (m, 2H, -CH_2Ar), 2.83 (m, 1H, -H_3), 4.03 (dd, 1H, *J* = 6.3 Hz, *J* = 9.2 Hz, -H_5), 4.33 (dd, 1H, *J* = 7.0 Hz, *J* = 9.2 Hz, -H_5), 5.94 (s, 2H, -O-CH_2-O-), 6.61 (dd, 1H, *J* = 1.7 Hz, *J* = 7.9 Hz, Ar), 6.63 (d, *J* = 1.7 Hz, Ar), 6.75 (d, 1H, *J* = 7.9 Hz, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 34.2, 37.3, 38.7, 72.5, 101.1, 108.5, 108.9, 121.6, 131.9, 146.4, 148.0, 176.8.

*6.1.6. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-(hydroxy(4-methoxyphenyl)methyl)-dihydrofuran-2(3H)-one (**6a**)*

A solution of compound **13** (70 mg, 0.3 mmol) in dry THF (0.5 ml) was added to a solution of LDA (0.4 ml, 0.6 mmol) in THF (3 ml) at − 78 °C and stirred for 30 min. To this solution 4-methoxybenzaladehyde (49 mg, 0.36 mmol) and HMPA (0.1 ml, 0.6 mmol) were added and the mixture was stirred for 2 h, then quenched with saturated aq. NH_4Cl (2 ml). The mixture was extracted with EtOAc (3 × 20 ml). The extract was washed successively with water (3 ml), 10% HCl (2 ml), water, saturated aq. $NaHCO_3$ and NaCl. Organic phase was dried (Na_2SO_4), filtered and evaporated *in vacuo* to give a brown viscous oil. The residue was purified by thick-layer chromatography (silica gel, hexane/EtOAc, 5:1) to give **6a** (70 mg, 65%) as mixture of diasteromers (*anti*/*syn*: 42/58); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01 $_{anti}$ (dd, *J* = 4.9 Hz, *J* = 13.6 Hz, -H_6), 2.13$_{anti}$ (dd, *J* = 9.8 Hz, *J* = 13.6 Hz, -H_6), 2.28 $_{syn}$ (dd, *J* = 7.0 Hz, *J* = 13.7 Hz, -H_6), 2.37 $_{syn}$ (dd, *J* = 4.5 Hz, *J* = 13.7 Hz, -H_6), 2.40 (brs, -OH), 2.46$_{anti}$ (m, -H_4), 2.62 $_{anti}$ (dd, *J* = 8.3 Hz, *J* = 8.6 Hz, -H_3), 2.65 $_{syn}$ (dd, *J* = 3.1 Hz, *J* = 8.6 Hz, -H_3), 2.82 $_{syn}$ (m, -H_4), 3.82 $_{syn}$ (s, 3H, -OMe), 3.89$_{anti}$ (s, -OMe), 3.89$_{anti}$ (t, *J* = 8.7 Hz, -H_5), 3.95 $_{syn}$ (dd, *J* = 6.2 Hz, *J* = 9.0 Hz, -H_5), 4.12$_{anti}$ (t, *J* = 8.5 Hz, -H_5), 4.17 (brs, -OH), 4.84 $_{anti}$ (d, *J* = 8.3 Hz, -H_7), 5.32 $_{syn}$ (s, *J* = 3.1 Hz, -H_7), 5.92 (s, 2H, -O-CH_2-O), 6.29–6.36 (m, 2H, Ar), 6.63 $_{syn}$ (d, *J* = 7.9 Hz, Ar), 6.68 $_{anti}$ (d, *J* = 8.1 Hz, Ar), 6.87$_{syn}$ (d, *J* = 8.7 Hz, Ph), 6.95$_{anti}$ (d, *J* = 8.6 Hz, Ph), 7.23$_{syn}$ (d, *J* = 8.7 Hz, Ph), 7.36$_{anti}$ (d, *J* = 8.6 Hz, Ph). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.5, 38.1, 39.3, 39.9, 51.5, 52.7, 55.3,71.9, 72.4, 74.3, 100.9, 108.1, 108.3, 108.7, 113.8, 114.1, 121.3, 121.5, 126.4, 127.9, 131.6, 132.8, 146.3, 147.8159.1, 159.9, 178.2, 179.1. FT-IR (KBr) ν 3456 (OH), 1763 (C=O) cm^{-1} (lactone). MS *m/z* (%): 356 (M^+, 14), 257 (6), 221 (8), 161 (10), 137 (100), 109 (16), 94 (12), 77 (20), 57 (12). Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.64; H, 5.39.

Other final compounds were prepared similarly, except **6g** which was synthesized from the reaction with benzyl bromide instead of benzaldehyde derivatives.

*6.1.7. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-(hydroxy(3-methoxyphenyl)methyl)-dihydrofuran-2(3H)-one (**6b**)*

Yield 70% as a mixture of diasteromers (*anti*/*syn*: 53/47); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01 $_{anti}$ (dd, *J* = 4.9 Hz, *J* = 13.8 Hz, -H_6), 2.16$_{anti}$ (dd, *J* = 9.9 Hz, *J* = 13.8 Hz, -H_6), 2.22 $_{syn}$ (dd, *J* = 6.9 Hz, *J* = 13.7 Hz, -H_6), 2.37 $_{syn}$ (dd, *J* = 8.7 Hz, *J* = 13.7 Hz, -H_6), 2.47$_{anti}$ (m, -H_4), 2.62 $_{anti}$ (dd, *J* = 8.3 Hz, *J* = 9.3 Hz, -H_3), 2.66 $_{syn}$ (dd, *J* = 2.9 Hz, *J* = 6.8 Hz, -H_3), 2.69 (brs, -OH), 2.84 $_{syn}$ (m, -H_4), 3.79 $_{syn}$ (s, -OMe), 3.82 $_{anti}$ (s, -OMe), 3.89$_{anti}$ (t, *J* = 9.0 Hz, -H_5), 3.94 $_{syn}$ (dd, *J* = 6.3 Hz, *J* = 8.9 Hz, -H_5), 4.13$_{anti}$ (t, *J* = 9.0 Hz, -H_5), 4.15 (brs, -OH), 4.32$_{syn}$(dd, *J* = 8.1 Hz, *J* = 8.9 Hz, -H_7), 4.85 $_{anti}$ (d, *J* = 8.3 Hz, -H_7), 5.35 $_{syn}$ (s, -H_7), 5.90 and 5.91 (2s, 2H, -O-CH_2-O), 6.27–34 (m, 2H, Ar), 6.61 (d, *J* = 7.8 Hz, Ar), 6.66 (d, *J* = 8.4 Hz, Ar), 6.82–6.99 (m, Ph), 7.25–7.34 (m, Ph). FT-IR (KBr) ν 3447 (OH), 1763 cm^{-1} (lactone). MS *m/z* (%): 356 (M^+, 10), 310 (4), 220 (25), 161 (19), 135 (100), 109 (30), 91 (23), 77 (30), 57 (31). Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.35; H, 5.72.

*6.1.8. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-(hydroxyl (3,4,5-trimethoxyphenyl) methyl)-dihydrofuran-2(3H)-one (**6c**) [21]*

Yield 74% as a mixture of diasteromers (*anti*/*syn*: 50/50); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.17$_{anti}$ (dd, *J* = 5.2 Hz, *J* = 13.9 Hz, -H_6), 2.25$_{anti}$ (dd, *J* = 9.5 Hz, *J* = 13.9 Hz, -H_6), 2.30 $_{syn}$ (dd, *J* = 8.1 Hz, *J* = 13.8 Hz, -H_6), 2.46 $_{syn}$ (dd, *J* = 7.8 Hz, *J* = 13.8 Hz, -H_6), 2.50 and 2.60 (m, -H_3), 2.64 $_{syn}$ (d, *J* = 8.6 Hz, -H_3), 2.68 (brs, -OH), 2.82 $_{anti}$ (dd, 1H, *J* = 7.2 Hz, *J* = 8.2 Hz, -H_3), 3.84 (s, 6H, -OMe), 3.89 (s, 3H,

-OMe), 3.92$_{anti}$ (t, 1H, J = 8.5 Hz, -H$_5$), 3.98 $_{syn}$ (dd, J = 7.0 Hz, J = 9.0 Hz, -H$_5$), 4.09 (brs, -OH), 4.17$_{anti}$ (t, J = 8.5 Hz, -H$_5$), 4.39$_{syn}$ (d, J = 8.2 Hz, -H$_7$), 4.81 $_{anti}$ (d, J = 8.0 Hz, -H$_7$), 5.26 $_{syn}$ (s, -H$_7$), 5.92 and 5.95 (s, 1H, -O-CH$_2$-O), 6.23 (s, Ph), 6.33 (m, Ph), 6.48 (s, Ph), 6.60 (d, J = 7.8 Hz, Ar), 6.65 (m, Ph). FT-IR (KBr) ν 3437 (OH), 1771 cm^{-1} (lactone). MS *m/z* (%): 416 (M$^+$, 26), 398 (5), 356 (4), 279 (4), 257 (18), 243 (11), 221 (15), 197 (100), 169 (52), 150 (28), 137 (49), 123 (15), 109 (17), 84 (58), 57 (38). Anal. Calcd for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.25; H, 4.96.

6.1.9. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-((S)-hydroxy (4-nitrophenyl)methyl)- dihydrofuran-2(3H)-one (**6d**)

Yield 65% as a mixture of diasteromers (*syn*/*anti*: 15/85), *syn* diasteromer:^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.05 (dd, 1H, J = 9.0 Hz, J = 13.7 Hz, -H$_6$), 2.21 (dd, 1H, J = 9.0 Hz, J = 13.7 Hz, -H$_6$), 2.54 (m, 1H, -H$_4$), 2.67 (t, 1H, J = 8.1 Hz, -H$_3$), 3.92 (t, 1H, J = 8.0 Hz, -H$_5$), 4.03 (brs, 1H, -OH), 4.47 (t, 1H, J = 8.0 Hz, -H$_5$), 5.46 (brs, 1H, -H$_7$), 5.82 and 5.88 (2s, 2H, -O-CH$_2$-O), 6.03 (s, 1H, Ar), 6.33 (d, 1H, J = 7.5 Hz, Ar), 6.60 (d, 1H, J = 7.5 Hz, Ar), 7.37 (d, 2H, J = 8.2 Hz, Ph), 8.14 (d, 2H, J = 8.2 Hz, Ph). FT-IR (KBr) ν 3440 (OH), 1749 cm^{-1} (lactone). MS *m/z* (%): 371 (M$^+$, 8), 279 (20), 257 (85), 243 (42), 229 (21), 213 (21), 167 (35), 149 (100), 135 (52), 115 (47), 97 (34), 83 (55), 69 (100), 55 (85). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61. Found: C, 61.38; H, 4.78; *anti* diasteromer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.23 (dd, 1H, J = 6.0 Hz, J = 13.8 Hz, -H$_6$), 2.35 (dd, 1H, J = 8.9 Hz, J = 13.8 Hz, -H$_6$), 2.51 (m, 1H, -H$_4$), 2.69 (t, 1H, J = 8.1 Hz, -H$_3$), 3.96 (dd, 1H, J = 7.8 Hz, J = 9.2 Hz, -H$_5$), 4.02 (brs, 1H, -OH), 4.24 (dd, 1H, J = 8.2 Hz, J = 9.2 Hz, -H$_5$), 5.01 (d, 1H, J = 7.7 Hz, -H$_7$), 5.93 (d, 1H, J = 9.9 Hz, -O-CH$_2$-O), 6.29 (s, 1H, Ar), 6.39 (d, 1H, J = 7.9 Hz, Ar), 6.69 (d, 1H, J = 7.9 Hz, Ar), 7.59 (d, 2H, J = 8.7 Hz, Ph), 8.26 (d, 2H, J = 8.7 Hz, Ph). FT-IR (KBr) ν 3445 (OH), 1748 cm^{-1} (lactone). MS *m/z* (%): 371 (M$^+$, 10), 341 (10), 306 (10), 279 (17), 257 (87), 243 (42), 229 (21), 213 (21), 185 (15), 161 (35), 149 (95), 135 (52), 115 (49), 97 (38), 83 (59), 69 (100). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61. Found: C, 61.55; H, 4.45.

6.1.10. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-(hydroxy(2-nitrophenyl)methyl)-dihydrofuran-2(3H)-one (**6e**)

Yield 73% as a mixture of diasteromers (*anti*/*syn*: 35/65): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.12$_{anti}$ (dd, J = 7.3 Hz, J = 13.8 Hz, -H$_6$), 2.35$_{anti}$ (dd, J = 8.4 Hz, J = 13.8 Hz, -H$_6$), 2.38 $_{syn}$ (dd, J = 9.8 Hz, J = 13.6 Hz, -H$_6$), 2.49 $_{syn}$ (dd, J = 5.3 Hz, J = 13.6 Hz, -H$_6$), 2.77$_{anti}$ (m, -H$_4$), 2.80 $_{anti}$ (dd, J = 6.3 Hz, J = 8.0 Hz, -H$_3$), 2.83 $_{syn}$ (m, -H$_4$), 2.96 $_{syn}$ (dd, J = 2.6 Hz, J = 6.7 Hz, -H$_3$), 3.90$_{anti}$ (t, J = 8.1 Hz, -H$_5$), 3.95 $_{syn}$ (dd, J = 6.3 Hz, J = 8.8 Hz, -H$_5$), 4.30$_{anti}$ (t, J = 7.6 Hz, -H$_5$), 4.36$_{syn}$ (t, J = 8.5 Hz, -H$_5$), 5.30 (brs, -OH), 5.53 $_{anti}$ (d, J = 6.3 Hz, -H$_7$), 5.92 (2s, 2H, -O-CH$_2$-O), 6.05 $_{syn}$ (d, J = 2.6 Hz, -H$_7$), 6.20 $_{anti}$ (s, Ar), 6.25 $_{syn}$ (dd, J = 7.9 Hz, Ar), 6.45 $_{syn}$ (s, Ar), 6.55 $_{syn}$ (d, J = 7.9 Hz, Ar), 6.68 $_{anti}$ (d, J = 8.3 Hz, Ar), 7.48 (dd, 1H, J = 7.6 Hz, J = 8.0 Hz, Ph), 7.67 $_{anti}$ (t, 1H, J = 7.3 Hz, Ph), 7.73 $_{syn}$ (t, 1H, J = 7.9 Hz, Ph), 7.88 $_{anti}$ (d, J = 7.7 Hz, Ph), 7.93 $_{syn}$ (d, J = 8.5 Hz, Ph), 8.03 (m, 1H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.5, 38.3, 39.3, 39.3, 40.5, 50.5, 51.6, 67.9, 68.5, 72.0, 72.3, 101.0, 108.2, 108.4, 108.6, 113.8, 121.4, 121.5, 124.2, 125.2, 128.5, 128.7, 129.1, 129.6, 131.2, 133.4, 133.7135.8, 137.0, 146.5, 147.6, 177.5, 177.8. FT-IR (KBr) ν 3425 (OH), 1779 cm^{-1} (lactone). MS *m/z* (%): 371 (M$^+$, 10), 341 (10), 306 (10), 269 (12), 257 (20), 236 (25), 219 (25), 185 (12), 161 (30), 149 (38), 137 (30), 111 (30), 97 (30), 91 (80), 69 (100), 55 (85). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61. Found: C, 61.62; H, 4.56.

6.1.11. (3S,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-(hydroxy(2-chlorophenyl)methyl)-dihydrofuran-2(3H)-one (**6f**)

Yield 70% as a mixture of diasteromers (*syn*/*anti*: 38/62), *syn* diasteromer:^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.09 (dd, 1H, J = 6.1 Hz, J = 13.7 Hz, -H$_6$), 2.35 (dd, 1H, J = 9.0 Hz, J = 13.7 Hz, -H$_6$), 2.63 (m, 1H, -H$_4$), 2.85 (dd, 1H, J = 7.2 Hz, J = 8.2 Hz, -H$_3$), 3.00 (brs, 1H, -OH), 3.94 (t 1H, J = 8.5 Hz, -H$_5$), 4.33 (t 1H, J = 8.5 Hz, -H$_5$), 5.70 $_{syn}$ (s, 1H, -H$_7$), 5.90 (s, 1H, -O-CH$_2$-O), 6.24 (s, 1H, Ar), 6.29 (d, 1H, J = 7.7 Hz, Ar), 6.60 (d, 1H, J = 7.7 Hz, Ar), 7.29 (m, 3H, Ph), 7.37 (m, 1H, Ph), 7.74 (d, 1H, J = 7.5 Hz, Ph). *anti* diasteromer: 2.09 (dd, 1H, J = 5.1 Hz, J = 13.8 Hz, -H$_6$), 2.18 (dd, 1H, J = 9.9 Hz, J = 13.8 Hz, -H$_6$), 2.64 (m, 1H, -H$_4$), 2.83 (m, 1H, -H$_3$), 3.01 (brs, 1H, -OH), 3.90 (t, 1H, J = 8.8 Hz, -H$_5$), 4.26 (t, 1H, J = 8.8 Hz, -H$_5$), 5.45 (d, J = 8.4 Hz, -H$_7$), 5.92 (s, 1H, -O-CH$_2$-O), 6.37 (d, 1H, J = 7.8 Hz, Ar), 6.40 (s, 1H, Ar), 6.68 (d, 1H, J = 7.8 Hz, Ar), 7.29 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.43 (d, 1H, J = 7.7 Hz, Ph), 7.65 (d, 1H, J = 7.7 Hz, Ph). FT-IR (KBr) ν 3456 (OH), 1744 cm^{-1} (lactone). MS *m/z* (%): 362 (M$^+$+2, 6), 361 (M$^+$+1, 10), 360 (M$^+$, 30), 284 (9), 257 (8), 220 (21), 191 (10), 165 (18), 135 (100), 115 (15), 85 (30), 77 (48), 57 (23). Anal. Calcd for $C_{19}H_{17}ClO_5$: C, 63.25; H, 4.75. Found: C, 63.48; H, 4.69.

6.1.12. (+) (3R,4R)-4-(Benzo[d][1,3]dioxol-5-ylmethyl)-3-benzyl-dihydrofuran-2(3H)-one (**6g**)

$[\alpha]_D^{25}$ + 4.8° (c = 0.0013, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.24 (dd, 1H, J = 6.0 Hz, J = 12.9 Hz, -H$_6$), 2.46 (m, 1H, -H$_4$), 2.56 (sextet, 1H, J = 5.1, Hz, J = 7.5 Hz, J = 10.7 Hz, -H$_3$), 2.95 (dd, 1H, J = 7.5 Hz, J = 14.0 Hz, -H$_7$), 3.13 (dd, 1H, J = 5.1 Hz, J = 14.0 Hz, -H$_7$), 3.86 (dd, 1H, J = 7.8 Hz, J = 9.2 Hz, -H$_5$), 4.10 (dd, 1H, J = 7.3 Hz, J = 9.2 Hz, -H$_5$), 5.95 (s, 1H, -O-CH$_2$-O), 6.44 (m, 2H, Ar), 6.69 (d, 1H, J = 8.3 Hz, Ar), 7.19 (d, 2H, J = 7.0 Hz, Ph), 7.24 (m, 1H, Ph), 7.324 (m, 2H, Ph). FT-IR (KBr) ν 1774 cm^{-1} (C═O). MS *m/z* (%): 310 (M$^+$, 54), 284 (10), 207 (11), 162 (14), 135 (100), 105 (15), 91 (24), 77 (19), 57 (12). Anal. Calcd for $C_{14}H_{18}O_4$: C, 73.53; H, 5.85. Found: C, 73.64; H, 5.66.

6.2. Cytotoxic assay [22]

Breast cancer human cell lines including MDA-MB 231, MCF-7 and T47D (human breast duct carcinoma; ATCC HTB-133 estrogen receptor-positive, ER$^+$) was obtained from Pasture institute, Tehran (Iran). Cells were maintained in RPMI 1640 with added 10% FBS, 1% L-Glutamine, and Penicillin-Streptomycin, and then cells were incubated at 37 °C in a 5% concentration of CO_2. Cells were harvested by Trypsin-EDTA and re-suspended in fresh medium. Cell survival was determined by MTT colorimetric assay. Exponentially growing cells (4×10^4 cells/well) were seeded in 96-well plates in RPMI with 10% FBS and incubated for 24 h. After treatment of cells with different concentrations of test compounds for 24 h at 37 °C, the medium was removed and phenol red-free medium with FBS was added to cells. Then MTT solution was added to each well (2 mg/ml), followed by 4 h incubation. The viable cell number is directly proportional to the production of formazan, which, following solubilization with isopropyl alcohol, can be measured spectrophotometrically at 492 nm by an ELISA plate reader. In each plate, there were control wells (cells without test compounds) and blank wells (the medium only or 0.1% DMSO). The percentage of cell viability versus controls was assessed by the formula [1 − (absorbance of treated cells/absorbance of control cells)] × 100.

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