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2,3-Dibenzylbutyrolactones and 1,2,3,4-Tetrahydro-2-Naphthoic acid γ-Lactones: Structure and Activity Relationship in Cytotoxic Activity

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Dibenzyl-g-butyrolactone and 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (TNL) derivatives were synthesized and evaluated for cytotoxic activity against some cancer cell lines. It was found that TNL derivatives with a shorter distance between C-4 in ring A and C'-2 in ring C were more cytotoxic, while dibenzyl-γ-butyrolactones with a longer one were nearly inactive. In TNL series, presence of 3,4-dioxy group in ring A and 2-methoxy group in ring C was essential for the enhancement of the activity.

Key words: Yatein analogues, Deoxypodophyllotoxin analogues, Cytotoxic activity

INTRODUCTION

(-)-Yatein [2-(3,4,5-trimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone], a well known naturally occurring lignan, was first isolated from *Juniperus thurifera*, showed a noticeable cytotoxic activity against some cancer cell lines (Medarde *et al.*, 1994).

Meanwhile, (-)-3-hydroxymethyl-6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (deoxypodophyllotoxin), the ring-closed form of the (-) yatein structure, was mainly isolated in the plants such as *Anthricus sylvestris* (Noguchi *et al.*, 1940), *Busera microphylla* (Bianchi *et al.*, 1968) and *Pulsatilla koreana* (Kim *et al.*, 2002), and found to show a potent cytotoxic activity against a variety of cancer cell lines. Its mode of action is the inhibition of microtubule assembly through binding to tubulin, which is an important target in the development of novel anticancer drugs (Jardine *et al.*, 1980). Currently, it was reported that deoxypodophyllotoxin also exhibited inhibitory effect on the tube formation of human umberical veinous endothelial (HUVE) cells (Kim *et al.*, 2002), Although various chemical modifications of natural lignans such as DPT, yatein have been made, they are directed to substituents and functionalities on the basic carbon frame (Terada *et al.*, 1992). Herein, we report on synthesis of yatein and deoxypodophylltoxin analogues and their structure-activity relationship towards cytotoxicity.

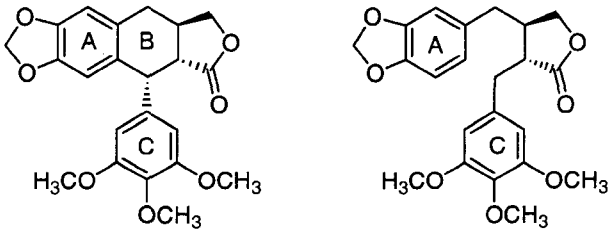

Fig. 1. Structure of deoxypodophyllotoxon and (-) yatein

MATERIALS AND METHODS

The IR spectra were recorded on a Jasco Report-100 FT-IR spectrometer, and only the principal bands were described. The ^{1}H-NMR spectra were recorded on a JEOL 90 MHz and 300 MHz spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Elemental analyses were performed using the Elementar Analysensystem GmbH Vario EL at the Seoul Branch Analytical Lab., Korea Basic Science Institute (KBSI) and within 0.4% of the theoretical values, unless otherwise noted. Column chromatography was monitored routinely by thin layer chromatography (TLC) on plates precoated with silica gel

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60 F254 from Merck. Silica gel 60 (70-230 mesh, E. Merck) was used for the column chromatography. All reagents, which were obtained commercially, were used without further purification, unless otherwise stated.

Synthesis

3,4-Methylenedioxybenzaldehyde *bis*(phenylthio) acetal (2a) 3,4-Methylenedioxybenzaldehyde (15 g, 0.1 mole) was dissolved in dry $CHCl_3$ (60 mL) and the solution was cooled to 0 °C. Thiophenol (20.5 mL, 0.2 mole) was then added and dry HCl gas was bubbled through the cooled reaction mixture for 1 h. After being stirred for a further 1 h, the solution was treated with 4 M NaOH and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, filtered, and evaporated to give the colorless oil. Purification of the product by column chromatography on a silica gel yielded colorless oil (32.4 g). Yield: 92%, ^{1}H NMR ($CDCl_3$): δ 7.36-7.21 (m, 10H), 6.97 (d, *J* = 1.74 Hz, 1H) 6.77 (dd, *J* = 1.74, 8.02 Hz, 1H), 6.64 (d, *J* = 8.02 Hz, 1H) 5.92 (s, 2H), 5.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 147.7, 147.3, 134.5, 133.4, 132.2, 128.7, 127.6, 121.4, 108.1, 107.7, 101.0, 60.1.

General procedure for the preparation of benzyl bromides (B1-B10)

To a suspension of triphenylphosphine (30.8 mmole) in CH_3CN (7 mL) maintained under nitrogen in an ice bath was added dropwise bromine (30.8 mmole) over a period of 20 min. The ice bath was removed, and after the addition of dry pyridine (2.2 mL), a solution of appropriate benzylalcohols (28 mmole) in CH_3CN (10 mL) was added in one portion. The reaction mixture was heated to 50°C for 30 min. The solvent was evaporated, and the resulting triphenylphosphine oxide was removed by column chromatography. The crude products were purified by column chromatography (silica gel, cyclohexane:EtOAc, 30:1).

3,4-dimethoxybenzyl bromide (B1) Yield: 70%, ^{1}H NMR ($CDCl_3$): δ 6.9-6.78 (m, 3H), 4.59 (s, 2H), 3.83 (s, 6H). IR ν_{max} cm^{-1} (neat) 2900, 1600, 1490, 1250.

2,3-Dimethoxybenzyl bromide (B2) Yield: 74%, ^{1}H NMR ($CDCl_3$): δ 7.05-6.92 (m, 3H), 4.58 (s, 2H), 3.97 (s, 3H), 3.85 (s, 3H), IR ν_{max} cm^{-1} (neat) 2950, 1600, 1490, 1250.

2,5-Dimethoxybenzyl bromide (B3) Yield: 66%, ^{1}H NMR ($CDCl_3$): δ 6.93 (s, 1H), 6.82 (s, 2H), 4.43 (s, 2H), 3.82 (s, 3H), 3.86 (s, 3H), IR ν_{max} cm^{-1} (neat) 2900, 1590, 1490, 1250.

2,5-Dibenzyloxybenzyl bromide (B4) Yield: 67%, ^{1}H NMR ($CDCl_3$): δ 7.38-6.96 (m, 13H), 5.08 (s, 2H), 5.01 (s, 2H), 4.40 (s, 2H), IR ν_{max} cm^{-1} (neat) 2950, 1590, 1490, 1250.

3,4-Methylenedioxybenzyl bromide (B5) Yield: 68%, ^{1}H NMR ($CDCl_3$): δ 6.96 (d, *J* = 1.78 Hz, 1H), 6.77 (dd, *J* = 1.78, 8.0 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 5.92 (s, 2H), 4.40 (s, 2H). IR ν_{max} cm^{-1} (neat) 2950, 1590, 1500, 1250.

2-Methoxybenzyl bromide (B6) Yield: 60%, ^{1}H NMR ($CDCl_3$): δ 7.37-6.82 (m, 4H), 4.56 (s, 2H), 3.88 (s, 3H), IR ν_{max} cm^{-1} 2950, 1590, 1490, 1250.

3-Methoxybenzyl bromide (B7) Yield: 65%, ^{1}H NMR ($CDCl_3$): δ 7.06-6.88 (m, 4H), 4.55 (s, 2H), 3.89 (s, 3H). IR ν_{max} cm^{-1} 2900, 1590, 1490, 1250.

4-Methoxybenzyl bromide (B8) Yield: 62%, ^{1}H NMR ($CDCl_3$): δ 7.07 (d, *J* = 8.70 Hz, 2H), 6.83 (d, *J* = 8.70 Hz, 2H), 4.56 (s, 2H), 3.90 (s, 3H), IR ν_{max} cm^{-1} 2950, 1590, 1490, 1250.

4-Biphenylbenzyl bromide (B9) Yield: 62%, ^{1}H NMR ($CDCl_3$) δ 7.68-7.31 (m, 9H), 4.53 (s, 2H), IR ν_{max} cm^{-1} 2950, 1590, 1490, 1260.

3,4,5-Trimethoxybenzyl bromide (B10) Yield: 59%, m.p. 75-77°C, ^{1}H NMR ($CDCl_3$): δ 6.62 (s, 2H), 4.47 (s, 2H), 3.87 (s, 3H), 3.85 (s, 6H), IR ν_{max} cm^{-1} 2950, 1595, 1490, 1260.

General procedure for the preparation of trans-2,3-dibenzyl-γ-butyrolactones (5a-5j)

To a stirred solution of dithioacetal **2** (5 mmole) in dry THF (80 mL) maintained nitrogen at -78°C was added a solution of n-butyllithium (3.13 mL, 1.6 M) in hexane. The resulting solution was stirred for 2 h and a solution of 2-butenolide (6.65 mmole) in dry THF (3 mL) was added. The reaction mixture was again stirred for 2 h at -78°C and then treated dropwise with a solution of benzylbromides (5 mmole) and hexamethylenephosphoramide (HMPA) (5 mmole) in dry THF (8 mL) and was allowed to slowly warm to room temperature for 3 h and then quenched with water. The mixture was extracted with ethyl acetate and extracts was washed with water. Evaporation of the solvent left an orange gum which was purified by silica gel column chromatography (cyclohexane : ethyl acetate = 20 : 1 → 5 : 1) to give the intermediates. Two spoonfuls (a larger excess) of Raney Ni (20 g) were added to a solution of the intermediates (3.5 mmole) in dry EtOH (30 mL). The resulting suspension was refluxed for 5 h, after which the reaction mixture was allowed to cool and then filtered through Celite, and the filter was washed with dry ethanol. The resulting filterate was concentrated in vacuo to give a colorless gum, which was purified by silicagel column chromatography (cyclohexane : ethyl acetate = 4 : 1).

(±)-2-(3,4-Dimethoxybenzyl)-3-(3,4-methylenedioxybenzy)-γ-butyrolactone (5a) Yield: 52 %. ^{1}H NMR ($CDCl_3$): δ 6.80-6.44 (m, 6H), 5.93 (d, *J* = 1.37 Hz, 1H), 5.92 (d, *J* = 1.37 Hz, 1H), 4.12 (dd, *J* = 6.84, 11.7 Hz, 1H), 3.87-3.84 (m, 7H), 3.00-2.85 (m, 2H), 2.63-2.44 (m, 4H).

IR ν_{max} cm^{-1} 2950, 1755, 1490, 1250.

(±)-2-(2,3-Dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5b) Yield: 46%, ^1H NMR ($CDCl_3$): δ 7.03-6.97 (m, 1H), 6.84-6.80 (m, 2H), 6.65 (d, *J* = 7.70 Hz, 1H), 6.43 (s, 1H), 6.40 (d, *J* = 7.70 Hz, 1H), 5.90 (s, 2H), 4.10 (dd, *J* = 7.25, 9.07 Hz, 1H), 3.87 (s, 3H), 3.85-3.79 (m, 4H), 3.28-2.28 (m, 6H). IR ν_{max} cm^{-1} 2900, 1750, 1495, 1250.

(±)-2-(2,5-Dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5c) Yield: 44%, ^1H NMR ($CDCl_3$): δ 6.80-6.38 (m, 6H), 5.90 (s, 2H), 4.12 (dd, *J* = 7.20, 9.10 Hz, 1H), 3.84-3.79 (m, 4H), 3.75 (s, 3H), 3.23 (dd, *J* = 5.04, 13.5 Hz, 1H), 2.84-2.29 (m, 5H). IR ν_{max} cm^{-1} 2955, 1750, 1485, 1250.

(±)-2-(2,5-Dihydroxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5d) Yield: 43%, ^1H NMR ($CDCl_3$): δ 9.15 (s, 1H), 9.13 (s, 1H), 6.77-6.41 (m, 6H), 5.90 (s, 2H), 4.15 (dd, *J* = 7.25, 9.15 Hz, 1H), 3.89-3.71 (m, 7H), 3.27-2.31 (m, 6H). IR ν_{max} cm^{-1} 3300, 2950, 1760, 1500.

(±)-2-(3,4-Methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5e) Yield: 47%. ^1H NMR ($CDCl_3$): δ 6.74-6.44 (m, 6H), 5.94-5.92 (m, 4H), 4.12 (dd, *J* = 4.20, 10.4 Hz, 1H), 3.85 (dd, *J* = 6.60, 10.4 Hz, 1H), 3.02-2.39 (m, 6H). IR ν_{max} cm^{-1} 2955, 1760, 1500, 1290.

(±)-2-(2-Methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5f) Yield: 52%, ^1H NMR ($CDCl_3$): δ 7.27-7.13 (m, 2H), 6.94-6.85 (m, 2H), 6.65 (d, *J* = 7.20 Hz, 1H), 5.90 (s, 2H), 4.12 (dd, *J* = 7.34, 9.00 Hz, 1H), 3.85-3.79 (m, 4H), 3.29 (dd, *J* = 5.01, 14.3 Hz, 1H), 2.84-2.27 (m, 5H). IR ν_{max} cm^{-1} 2950, 1760, 1490, 1250.

(±)-2-(3-Methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5g) Yield: 53%, ^1H NMR ($CDCl_3$): δ 7.26-7.19 (m, 1H), 6.79-6.67 (m, 4H), 6.45-6.43 (m, 2H), 5.92 (s, 2H), 4.11 (dd, *J* = 7.03, 9.01 Hz, 1H), 3.84 (dd, *J* = 7.32, 9.01 Hz, 1H), 3.78 (s, 3H), 3.06 (dd, *J* = 4.98, 13.8 Hz, 1H), 2.88 (dd, *J* = 7.39, 13.9 Hz, 1H), 2.61-2.38 (m, 4H). IR ν_{max} cm^{-1} 2900, 1745, 1490, 1250.

(±)-2-(4-Methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5h) Yield: 47%, ^1H NMR ($CDCl_3$): δ 7.08 (d, *J* = 8.70 Hz, 1H), 6.83 (d, *J* = 8.70 Hz, 1H), 6.69 (d, *J* = 8.11 Hz, 1H), 6.47-6.44 (m, 3H), 5.92 (s, 2H), 4.07 (dd, *J* = 6.90, 9.75 Hz, 1H), 3.82 (dd, *J* = 7.12, 9.75 Hz, 1H), 3.78 (s, 3H), 3.04-2.84 (m, 2H), 2.62-2.41 (m, 4H), IR ν_{max} cm^{-1} 2950, 1750, 1500, 1250.

(±)-2-(4-Phenylbenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (5e) Yield: 49%, ^1H NMR ($CDCl_3$): δ 7.59-7.21 (m, 9H), 6.68 (d, *J* = 8.28 Hz, 1H), 6.46-6.43 (m, 2H), 5.87 (d, *J* = 1.33 Hz, 1H), 5.84 (d, *J* = 1.33 Hz, 1H), 4.12 (dd, *J* = 7.03, 9.12 Hz, 1H), 3.85 (dd, *J* = 7.29, 9.12 Hz, 1H), 3.13-2.93 (m, 2H), 2.65-2.44 (m, 4H). IR ν_{max} cm^{-1} 2955, 1750, 1490, 1245.

(±)-Yatein (5j) Yield: 53%, ^1H NMR ($CDCl_3$): δ 6.69 (d, *J* = 8.39 Hz, 1H), 6.51-6.46 (m, 3H), 5.92 (d, *J* = 1.32 Hz, 1H), 5.91 (d, *J* = 1.32 Hz, 1H), 4.17 (dd, *J* = 7.13, 9.23 Hz, 1H), 3.86 (dd, *J* = 7.46, 9.23 Hz, 1H), 3.83 (s, 9H), 2.92-2.47 (m, 6H). IR ν_{max} cm^{-1} 2950, 1760, 1490, 1250.

General procedure for the preparation of (±)-trans-4-(α-hydroxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6a-6p)

To a stirred solution of dithioacetal **2** (5 mmole) in dry THF (80 mL) maintained nitrogen at -78°C was added a solution of butyllithium (3.13 mL, 1.6 M) in hexane. The resulting solution was stirred for 2 h and a solution of 2-butenolide (6.65 mmole) in dry THF (3 mL) was added. The reaction mixture was again stirred for 2 h at -78°C and added to a solution of benzaldehydes (5 mmole) in dry THF (8 mL). The reaction mixture was further stirred for 2 hr and then quenched with water. The mixture was extracted with ethyl acetate and extract was washed with water. Evaporation of the solvent left an orange gum which was purified by silicagel column chromatography (cyclohexane : ethyl acetate=10:1 → 3:1).

(±)-*trans*-4-(α-hydroxy-3,4-dimethoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6a) Yield: 74%, ^1H NMR ($CDCl_3$): δ 7.31-7.14 (m, 10H), 6.74-6.56 (m, 6H), 5.96 (d, *J* = 1.45 Hz, 1H), 5.94 (d, *J* = 1.45 Hz, 1H), 5.12 (d, *J* = 3.68 Hz, 1H), 4.62 (dd, *J* = 4.61, 9.71 Hz, 1H), 4.00 (dd, *J* = 8.10, 9.71 Hz, 1H), 3.87 (s, 3H), 3.77 (s, 3H), 3.39 (dd, *J* = 1.91, 5.15 Hz, 1H), 3.24 (dd, *J* = 2.70, 5.15 Hz, 1H), 2.95-2.85 (m, 1H). IR ν_{max} cm^{-1} 3480, 1760, 1470, 1250.

(±)-*trans*-4-(α-Hydroxy-2,3-dimethoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6b) Yield: 72%. ^1H NMR ($CDCl_3$): 7.51-6.60 (m, 16H), 5.94 (s, 2H), 5.46 (d, *J* = 3.40 Hz, 1H), 4.72 (dd, *J* = 1.97, 9.84 Hz, 1H), 4.15 (dd, *J* = 7.78, 9.84 Hz, 1H), 3.86 (s, 6H), 3.34-2.95 (m, 3H). IR ν_{max} cm^{-1} 3480, 1765, 1470, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6c) Yield: 76%, ^1H NMR ($CDCl_3$): δ 7.28-7.08 (m, 10H), 6.72-6.64 (m, 6H), 5.92 (s, 2H), 5.41 (d, *J* = 3.51 Hz, 1H), 4.72 (d, *J* = 9.75 Hz, 1H), 3.99 (dd, *J* = 7.68, 9.75 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.43 (t, *J* = 4.61 Hz, 1H), 2.87 (d, *J* = 7.90 Hz, 1H), 2.75 (d, *J* = 5.12 Hz, 1H). IR ν_{max} cm^{-1} 3480, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethylbenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6d) Yield: 68%, ^1H NMR ($CDCl_3$): δ 7.29-6.94 (m, 15H), 6.59 (d, *J* = 8.31 Hz, 1H), 5.90 (s, 2H), 5.39 (d, *J* = 2.30 Hz, 1H), 4.86 (d, *J* = 9.73 Hz, 1H), 4.35 (dd, *J* = 8.08, 9.73 Hz, 1H), 3.06-2.89 (m, 2H), 2.20 (s, 3H), 2.17 (s, 3H). IR ν_{max} cm^{-1} 3485, 1760, 1470, 1250.

(±)-*trans*-4-(α-Hydroxy-5-bromo-2-methoxybenzyl)-3-[3,4-Methylenedioxy-α,α-bis(phenylthio)-benzyl]-γ-butyrolactone (6e) Yield: 70%, ^{1}H NMR ($CDCl_3$): δ 7.40-7.0[illegible] (m, 14H), 6.65 (d, *J* = 7.51 Hz, 1H), 5.93 (s, 2H), 5.4[illegible] (dd, *J* = 2.77, 4.88 Hz, 1H), 4.86 (d, *J* = 9.57 Hz, 1H), 4.27 (dd, *J* = 8.05, 9.57 Hz, 1H), 3.80 (s, 3H), 3.43 (s, 1H), 2.8[illegible]-2.71 (m, 2H). IR ν_{max} cm^{-1} 3485, 1765, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-3,4-methylenedioxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)-benzyl]-γ-butyrolactone (6f) Yield: 68%, ^{1}H NMR ($CDCl_3$): δ 7.51-7.1[illegible] (m, 12H), 6.70-6.43 (m, 4H), 5.94 (s, 4H), 5.12 (d, *J* = 4.77 Hz, 1H), 4.64 (d, *J* = 10.0 Hz, 1H), 4.09 (dd, *J* = 8.05, 10.0 Hz, 1H), 3.17 (s, 1H), 2.94-2.85 (m, 2H). IR ν_{max} cm^{-1} 3485, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-2-methoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6g) Yield: 76%, ^{1}H NMR ($CDCl_3$): δ 7.29-7.06 (m, 17H), 5.93 (s, 2H), 5.48 (d, *J* = 3.15 Hz, 1H), 4.69 (dd, *J* = 1.59, 9.72 Hz, 1H), 4.19 (dd, *J* = 8.07, 9.72 Hz, 1H), 3.80 (s, 3H), 3.42 (t, *J* = 5.10 Hz, 1H), 2.75 (d, *J* = 7.44 Hz, 1H). IR ν_{max} cm^{-1} 3480, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-4-methoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6h) Yield: 74%, ^{1}H NMR ($CDCl_3$): δ 7.52-6.62 (m, 17H), 5.94 (s, 2H), 5.12 (d, *J* = 3.16 Hz, 1H), 4.61 (dd, *J* = 1.97, 9.48 Hz, 1H), 3.99 (dd, *J* = 8.05, 9.48 Hz, 1H), 3.79 (s, 3H), 3.23 (t, *J* = 5.63 Hz, 1H), 2.99-2.82 (m, 2H). IR ν_{max} cm^{-1} 3490, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-4-phenylbenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6i) Yield: 62%, m.p: 102-104°C, ^{1}H-NMR ($CDCl_3$): δ 7.53-6.60 (m, 22H), 5.91 (s, 2H), 5.25 (d, *J* = 3.41 Hz, 1H), 4.67 (dd, *J* = 1.85, 11.7 Hz, 1H), 4.11 (dd, *J* = 8.05, 11.7 Hz, 1H), 3.26 (t, *J* = 5.15 Hz, 1H), 2.97-2.88 (m, 2H). IR ν_{max} cm^{-1} 3480, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-3,4,5-trimethoxybenzyl)-3-[3,4-methylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6j) Yield: 62%, m.p: 102-104°C, ^{1}H-NMR ($CDCl_3$): δ 7.53-6.60 (m, 22H), 5.91 (s, 2H), 5.25 (d, *J* = 3.41 Hz, 1H) 4.67 (dd, *J* = 1.85, 11.7 Hz, 1H), 4.11 (dd, *J* = 8.05, 11.7 Hz, 1H), 3.26 (t, *J* = 5.15 Hz, 1H), 2.97-2.88 (m, 2H). IR ν_{max} cm^{-1} 3480, 1770, 1470, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-[2-methoxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6k) Yield: 78%, ^{1}H NMR ($CDCl_3$): δ 7.29-6.72 (m, 17H), 5.38 (d, *J* = 3.31 Hz, 1H), 4.85 (d, *J* = 9.83 Hz, 1H), 4.34 (dd, *J* = 7.98, 9.83 Hz, 1H), 3.75 (s, 3H), 3.69 (s, 3H), 3.42-2.74 (m, 4H). IR ν_{max} cm^{-1} 3480, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5,-dimethoxybenzyl)-3-[2.3-dimethoxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6l) Yield: 78%, ^{1}H NMR ($CDCl_3$): δ 7.39-6.71 (m, 16H), 5.56 (d, *J* = 3.22 Hz, 1H), 4.15-3.92 (m, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H), 3.72 (s, 3H), 3.43-2.75 (m, 4H). IR ν_{max} cm^{-1} 3475, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5,-dimethoxybenzyl)-3-[3,4-dimethoxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6m) Yield: 68%, ^{1}H NMR ($CDCl_3$): δ 7.26-7.07 (m, 12H), 6.80-6.65 (m, 4H), 5.54 (d, *J* = 3.25 Hz, 1H), 4.29 (dd, *J* = 2.95, 9.75 Hz, 1H), 3.90-3.80 (m, 4H), 3.75 (s, 3H), 3.73 (s, 3H), 3.60 (s, 3H), 3.40-2.58 (m, 4H). IR ν_{max} cm^{-1} 3470, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5,-dimethoxybenzyl)-3-[3,4,5-trimethoxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6n) Yield: 80%, ^{1}H NMR ($CDCl_3$) δ 7.29-7.16 (m, 10H), 6.88-6.71 (m, 5H), 5.46 (d, *J* = 2.74 Hz, 1H), 4.65 (dd, *J* = 2.74, 9.07 Hz, 1H), 4.21 (dd, *J* = 3.71, 9.07 Hz, 1H), 3.82 (s, 6H), 3.71 (s, 9H), 3.52-3.48 (m, 2H), 3.07-2.99 (m, 2H). IR ν_{max} cm^{-1} 3470, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5,-dimethoxybenzyl)-3-[2,5-dimethoxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (6m) Yield: 71%, ^{1}H NMR ($CDCl_3$): δ 7.31-6.58 (m, 16H), 5.53 (d, *J* = 3.12 Hz, 1H), 4.63 (dd, *J* = 2.75, 9.07 Hz, 1H), 4.19 (dd, *J* = 3.61, 9.07 Hz, 1H), 3.78 (s, 3H), 3.7 (s, 6H), 3.62 (s, 3H), 2.97-2.16 (m, 4H). IR ν_{max} cm^{-1} 3450, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-[3,4-ethylenedioxy-α,α-bis(phenylthio)benzyl]-γ-butyrolactone (16p) Yield: 75%, ^{1}H NMR ($CDCl_3$) δ 7.32-6.30 (m, 16H), 5.29 (d, *J* = 7.60 Hz, 1H), 4.29-4.11 (m, 5H), 3.93-3.82 (m, 4H), 3.78 (s, 3H), 3.43-2.17 (m, 4H). IR ν_{max} cm^{-1} 3460, 1765, 1490, 1250.

General procedure for the preparation of (±)-trans-2-(α-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactones (7a-7p)

(±)-trans-2-(α-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactones were synthesized from **6a** in a same manner to that described for **5a**.

(±)-*trans*-2-(α-Hydroxy-3,4-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (7a) Yield: 39 %, ^{1}H NMR ($CDCl_3$): δ 6.91-6.67 (m, 2H), 6.58 (d, *J* = 7.83 Hz, 1H), 6.33-6.21 (m, 3H), 5.91 (d, *J* = 1.50 Hz, 1H), 5.88 (d, *J* = 1.50 Hz, 1H), 5.27 (d, *J* = 2.12 Hz, 1H), 4.33 (dd, *J* = 7.99, 8.69 Hz, 1H), 3.93 (dd, *J* = 5.50, 8.67 Hz, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 2.89-2.22 (m, 5H). IR ν_{max} cm^{-1} 3470, 1760, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-2,3-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (7b) Yield: 36 %, ^{1}H NMR ($CDCl_3$): δ 7.17-6.27 (m, 6H), 5.86 (s, 2H), 5.58 (s, 1H), 4.17 (dd, *J* = 7.81, 8.71 Hz, 1H), 3.90-3.73 (m, 7H) 3.05-2.21 (m, 5H). IR ν_{max} cm^{-1} 3475, 1760, 1475, 1260.

(±)-*trans*-2-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (7c) Yield: 37 %, ^{1}H NMR ($CDCl_3$): δ 7.15-6.24 (m, 6H), 5.89 (s, 2H),

5.56 (s, 1H), 4.28 (dd, *J* = 8.04, 9.00 Hz, 1H), 3.89 (dd, *J* = 6.54, 9.00 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 2.93-2.11 (m, 5H). IR ν_{max} cm^{-1} (neat) 3470, 1765, 1475, 1250.

(±)-*trans*-2-(α-Hydroxy-2,5-dimethylbenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7d**) Yield: 47 %, ^{1}H NMR ($CDCl_3$): δ 7.44 (s, 1H), 6.97 (s, 2H), 6.56 (d, *J* = 8.05 Hz, 1H), 6.27-6.17 (m, 2H), 5.88 (s, 2H), 5.52 (d, *J* = 2.42 Hz, 1H), 4.35 (t, *J* = 16.9 Hz, 1H), 3.92 (dd, *J* = 6.08, 8.90 Hz, 1H). 2.98-1.99 (m, 12H). IR ν_{max} cm^{-1} 3475, 1760, 1470, 1290.

(±)-*trans*-2-(α-Hydroxy-5-bromo-2-methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7e**) Yield: 40%, ^{1}H NMR ($CDCl_3$): δ 7.59-6.29 (m, 6H), 5.87 (s, 2H), 5.58 (d, *J* = 4.77 Hz, 1H), 4.28 (dd, *J* = 7.51, 8.77 Hz, 1H), 3.87 (dd, *J* = 5.81, 8.77 Hz, 1H), 3.75 (s, 3H), 2.97-2.66 (m, 4H), 2.25-2.03 (m, 2H). IR ν_{max} cm^{-1} 3470, 1760, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-3,4-methylenedioxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7f**) Yield: 47%, ^{1}H NMR ($CDCl_3$): δ 6.69-6.26 (m, 6H), 5.93 (s, 2H), 5.89 (s, 2H), 5.49 (d, *J* = 4.87 Hz, 1H), 4.35 (dd, *J* = 7.87, 7.96 Hz, 1H), 3.92 (dd, *J* = 4.56, 7.96 Hz, 1H), 3.11-2.14 (m, 6H). IR ν_{max} cm^{-1} 3470, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-2-methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7g**) Yield: 45 %, ^{1}H NMR ($CDCl_3$): δ 7.55 (dd, *J* = 0.87, 7.51 Hz, 1H), 7.32-7.23 (m, 1H), 7.02 (t, *J* = 7.51, 8.16 Hz, 1H), 6.77 (d, *J* = 8.16 Hz, 1H), 6.57 (d, *J* = 8.16 Hz, 1H), 6.32-6.16 (m, 2H), 5.88 (s, 2H), 5.59 (s, 1H), 4.28 (dd, *J* = 8.13, 8.61 Hz, 1H), 3.88 (dd, *J* = 6.40, 8.61 Hz, 1H), 3.76 (s, 3H), 2.93-2.07 (m, 6H). IR ν_{max} cm^{-1} 3470, 1765, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-4-methoxybenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7h**) Yield: 43.8%, ^{1}H NMR ($CDCl_3$) δ 7.26-6.28 (m, 7H), 5.29 (s, 2H), 5.30 (d, *J* = 2.68 Hz, 1H), 4.61 (dd, *J* = 1.94, 7.60 Hz, 1H), 3.91 (dd, *J* = 5.64, 7.60 Hz, 1H), 3.80 (s, 3H), 2.92-2.2 (m, 6H). IR ν_{max} cm^{-1} 3470, 1770, 1470, 1250.

(±)-*trans*-2-(α-Hydroxy-4-phenylbenzyl)-3-(3,4-methylenedioxybenzyl)-γ-butyrolactone (**7i**) Yield: 45%, m.p: 95-97°C, ^{1}H NMR ($CDCl_3$): δ 7.66-7.32 (m, 9H), 6.62-6.25 (m, 3H), 5.76 (d, *J* = 1.25 Hz, 1H), 5.62 (d, *J* = 1.25 Hz, 1H), 5.38 (s, 1H), 4.27 (dd, *J* = 1.85, 11.7 Hz, 1H), 3.92 (dd, *J* = 5.72, 11.7 Hz, 1H), 2.88-2.15 (m, 6H). IR ν_{max} cm^{-1} 3470, 1765, 1470, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(2-methoxybenzyl)-γ-butyrolactone (**7k**) Yield: 37%, ^{1}H NMR ($CDCl_3$): δ 7.17-6.27 (m, 7H), 5.39 (s, 1H), 4.35 (d, *J* = 9.73 Hz, 1H), 3.84 (dd, *J* = 6.15, 9.73 Hz, 1H), 3.73 (s, 3H), 3.71 (s, 3H), 3.67 (s, 3H), 2.66-2.20 (m, 6H). IR ν_{max} cm^{-1} 3460, 1765, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(2,3-dimethoxybenzyl)-γ-butyrolactone (**7l**) Yield: 38%, ^{1}H NMR ($CDCl_3$): δ 7.27-6.36 (m, 6H), 5.60 (s, 1H), 4.15 (d, *J* = 5.81 Hz, 1H), 4.00-3.90 (m, 7H), 3.76 (s, 3H), 3.64 (s, 3H), 2.95-2.87 (m, 3H), 2.36-2.16 (m, 3H). IR ν_{max} cm^{-1} (neat) 3470, 1765, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(3,4-dimethoxybenzyl)-γ-butyrolactone (**7m**) Yield: 40%, ^{1}H NMR ($CDCl_3$): δ 7.29-6.42 (m, 6H), 5.56 (s, 1H), 4.40 (d, *J* = 6.06 Hz, 1H), 4.10-3.85 (m, 7H), 3.78 (s, 3H), 3.74 (s, 3H), 3.18-3.14 (m, 3H), 2.63-2.11 (m, 3H). IR ν_{max} cm^{-1} 3470, 1770, 1490, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(3,4,5-trimethoxybenzyl)-γ-butyrolactone (**7n**) Yield: 45%, ^{1}H NMR ($CDCl_3$) δ 7.17-6.58 (m, 3H), 5.99 (s, 2H), 5.51 (s, 1H), 4.41-4.31 (m, 1H), 4.03-3.87 (m, 1H), 3.78 (s, 9H), 3.77 (s, 3H), 3.65 (s, 3H), 2.94-2.81 (m, 3H), 2.39-2.17 (m, 3H). IR ν_{max} cm^{-1} 3470, 1770, 1500, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(2,5-dimethoxybenzyl)-γ-butyrolactone (**7o**) Yield: 37%, ^{1}H NMR ($CDCl_3$): δ 7.16-6.33 (m, 6H), 5.53 (s, 1H), 4.28-4.19 (m, 1H), 4.03-3.93 (m, 1H), 3.78 (s, 3H), 3.70 (s, 6H), 3.62 (s, 3H), 2.97-2.79 (m, 3H), 2.45-2.21 (m, 3H). IR ν_{max} cm^{-1} 3475, 1770, 1500, 1250.

(±)-*trans*-4-(α-Hydroxy-2,5-dimethoxybenzyl)-3-(3,4-ethylenedioxybenzyl)-γ-butyrolactone (**7p**) Yield: 45%, ^{1}H NMR ($CDCl_3$): δ 7.08-6.30 (m, 6H), 5.28 (d, *J* = 6.07 Hz, 1H), 4.29-4.11 (m, 5H), 3.93-3.82 (m, 4H), 3.78 (s, 3H), 2.66-2.58 (m, 3H), 2.17-2.10 (m, 3H). IR ν_{max} cm^{-1} 3470, 1770, 1450, 1250.

General procedure for the preparation of 3-hydroxymethyl-6,7-methylenedioxy-1-(phenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8a-8p)

To a solution of **7a** (1.2 mmole) in dry dichloromethane (10 mL) was added a trifluoroacetic acid (1 mL). The resulting solution was stirred for 2 hr at ambient temperature and then poured into water (100 mL). The mixture was extracted with dichloromethane (100 mL×3), and the organic layer was combined and concentrated in vacuo. The crude product was purified by column chromatography with cyclohexane:ethyl acetate=3:1 on silica gel to give white solid.

3-Hydroxymethyl-6,7-methylenedioxy-1-(3,4-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (**8a**) Yield: 65%, m.p: 203-204.3°C, ^{1}H NMR ($CDCl_3$): δ 6.82 (s, 1H), 6.78 (d, *J* = 1.94 Hz, 1H), 6.70 (d, *J* = 1.94 Hz, 1H), 6.58 (s, 1H), 5.87 (d, *J* = 1.34 Hz, 1H), 5.85 (d, *J* = 1.34 Hz, 1H), 4.49 (dd, *J* = 6.20, 8.57 Hz, 1H), 4.06 (d, *J* = 10.4 Hz, 1H), 3.96 (dd, *J* =8.83, 10.4 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 2.95-2.47 (m, 4H). IR ν_{max} cm^{-1} 2900, 1760, 1450, 1250.

3-Hydroxymethy-6,7-methylenedioxy-1-(2,3-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (**8b**) Yield: 93%, m.p: 247-249°C, ^{1}H NMR ($CDCl_3$) δ

6.84 (s, 1H), 6.78 (d, *J* = 1.90 Hz, 1H), 6.70 (d, *J* = 1.90 Hz, 1H), 6.58 (s, 1H), 5.87 (d, *J* = 1.34 Hz, 1H), 5.85 (d, *J* = 1.34 Hz, 1H), 4.49 (dd, *J* = 6.20, 8.57 Hz, 1H), 4.06 (d, *J* = 10.4 Hz, 1H), 3.96 (dd, *J* = 8.83, 10.4 Hz, 1H), 3.86 (s, 3H) 3.83 (s, 3H), 2.95-2.47 (m, 4H). IR ν_{max} cm^{-1} 2950, 1770, 1490, 1250.

3-Hydroxymethyl-6,7-methylenedioxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8c) Yield: 93%, m.p: 155-157°C, ^{1}H NMR ($CDCl_3$): δ 6.86-6.30 (m, 6H), 5.87 (d, *J* = 1.29 Hz, 1H), 5.85 (d, *J* = 1.29 Hz, 1H), 5.13 (d, *J* = 6.13 Hz, 1H), 4.44 (dd, *J* = 6.77, 9.32 Hz, 1H), 3.97-3.88 (m, 4H), 3.66 (s, 2H), 3.08-2.70 (m, 4H). IR ν_{max} cm^{-1} 2900, 1760, 1450,1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(2,5-dimethylphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8d) Yield: 81%, m.p.; 216-218.1°C, ^{1}H NMR ($CDCl_3$): δ 7.08 (d, *J* = 7.87 Hz, 1H), 6.96 (dd, *J* = 1.43, 7.87 Hz, 1H), 6.64 (s, 1H), 6.45 (s, 1H), 6.31 (s, 1H), 5.88 (s, 2H), 4.83 (d, *J* = 5.90 Hz, 1H), 4.49 (dd, *J* = 5.81, 8.45 Hz, 1H), 3.91 (t, *J* = 17.8 Hz, 1H), 3.06-2.68 (m, 7H), 2.15 (s, 3H), IR ν_{max} cm^{-1} 2910, 1755, 1450, 1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(5-bromo-2-methoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8e) Yield: 79%, m.p.; 189-191°C, ^{1}H NMR ($CDCl_3$): δ 7.26-6.58 (m, 4H), 6.41 (s, 1H), 5.86 (s, 2H), 5.19 (d, *J* = 5.37 Hz, 1H), 4.43 (dd, *J* = 5.91, 8.55 Hz, 1H), 3.93-3.76 (m, 4H), 3.00-2.69 (m, 4H), IR ν_{max} cm^{-1} 2900, 1755, 1450, 1260

3-Hydroxymethyl-6,7-methylenedioxy-1-(3,4-methylenedioxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8f) Yield: 88%, m.p: 248-250°C, ^{1}H NMR ($CDCl_3$): δ 6.75 (s, 2H), 6.60 (s, 2H), 6.24 (s, 1H), 5.94 (s, 2H), 5.87 (s, 2H), 5.09 (d, *J* = 5.07 Hz, 1H), 4.46 (dd, *J* = 5.57, 8.75 Hz, 1H), 3.98 (dd, *J* = 5.41, 8.75 Hz, 1H), 2.99-2.61 m, 4H), IR ν_{max} cm^{-1} 2900, 1760, 1450, 1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(2-methoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8g) Yield: 94%, m.p: 145-147°C, ^{1}H NMR ($CDCl_3$): δ 6.94-6.73 (m, 4H), 6.63 (s, 1H), 6.42 (s, 1H), 5.87 (s, 2H), 5.20 (d, *J* = 5.17 Hz, 1H), 4.47 (dd, *J* = 5.58, 8.75 Hz, 1H), 4.00-3.88 (m, 4H), 2.93-2.59 (m, 4H), IR ν_{max} cm^{-1} 2900, 1760, 1455, 1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(4-methoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8h) Yield: 85%, m.p: 144-147.2°C, ^{1}H NMR ($CDCl_3$): δ 7.12 d, *J* = 8.40 Hz, 2H), 6.86 (d, *J* = 8.40 Hz, 2H), 6.58 (s, 1H), 6.29 (s, 1H), 5.86 (s, 2H), 4.49 (dd, *J* = 6.11, 8.40 Hz, 1H), 4.06 (d, *J* = 10.7 Hz, 1H), 3.95 (dd, *J* = 9.01, 10.7 Hz, 1H), 3.79 (s, 3H), 2.99-2.48 (m, 4H), IR ν_{max} cm^{-1} 2950, 1760, 1455, 1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(4-biphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8i) Yield: 86%, m.p: 249-252.6°C, ^{1}H NMR ($CDCl_3$): δ 7.65-7.31 (m, 9H), 6.62 (s, 1H), 6.39 (s, 1H), 5.75 (d, *J* = 1.15 Hz, 1H), 5.61 (d, *J* = 1.15 Hz, 1H), 5.31 (s, 1H), 4.28 (dd, *J* = 1.85, 11.7 Hz, 1H), 3.93 (dd, *J* = 5.72, 11.7 Hz, 1H), 2.88-2.15 (m, 4H), IR ν_{max} cm^{-1} 2950, 1760, 1455, 1260.

3-Hydroxymethyl-6,7-methylenedioxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8j) The spectroscopic data (IR, NMR) were identical to those reported (Medarde *et al.,* 1995).

3-Hydroxymethyl-5-methoxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8k) Yield: 73%, m.p: 169-171°C, ^{1}H NMR ($CDCl_3$): δ 7.11-6.29 (m, 6H), 5.07 (d, *J* = 5.05 Hz, 1H), 4.59 (dd, *J* = 6.10, 9.17 Hz, 1H), 3.95-3.85 (m, 4H), 3.01-2.60 (m, 4H), IR ν_{max} cm^{-1} 2900, 1755, 1450, 1260.

3-Hydroxymethyl-5,6-dimethoxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8l) Yield: 72%, m.p: 158-160°C, ^{1}H NMR ($CDCl_3$): δ 6.99-6.27 (m, 5H), 5.25 (d, *J* = 5.66 Hz, 1H), 4.51 (dd, *J* = 5.99, 8.99 Hz, 1H), 3.93-3.85 (m, 7H), 2.99-2.61 (m, 4H), IR ν_{max} cm^{-1} 2900, 1750, 1450, 1260.

3-Hydroxymethyl-6,7-dimethoxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8m) Yield: 65%, m.p: 154-156.3°C, ^{1}H NMR ($CDCl_3$): δ 6.89-6.25 (m, 5H), 5.26 (d, *J* = 5.55 Hz, 1H), 4.45 (dd, *J* = 5.81, 9.01 Hz, 1H), 3.99-3.91 (m, 7H), 2.89-2.51 (m, 4H), IR ν_{max} cm^{-1} 2910, 1755, 1450, 1260.

3-Hydroxymethyl-5,6,7-trimethoxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8n) Yield: 74%, m.p: 157.2-158.6°C, ^{1}H NMR ($CDCl_3$): δ 6.93-6.27 (m, 4H), 5.35 (d, *J* = 5.63 Hz, 1H), 4.46 (dd, *J* = 6.08, 9.07 Hz, 1H), 3.94-3.86 (m, 4H), 3.85 (s, 3H), 3.77 (s, 3H), 3.72 (s, 3H), 3.64 (s, 3H), 3.09-2.59 (m, 4H), IR ν_{max} cm^{-1} 2910, 1755, 1450, 1250.

3-Hydroxymethyl-5,8-dimethoxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8o) Yield: 76%, m.p: 158.2-159.6°C, ^{1}H NMR ($CDCl_3$): δ 6.91-6.26 (m, 5H), 5.21 (d, *J* = 5.61 Hz, 1H), 4.44 (dd, *J* = 6.10, 9.11 Hz, 1H), 3.95-3.87 (m, 4H), 3.70 (s, 6H), 3.62 (s, 3H), 3.11-2.61 (m, 4H), IR ν_{max} cm^{-1} 2900, 1760, 1450, 1250.

3-Hydroxymethyl-6,7-ethylenedioxy-1-(2,5-dimethoxyphenyl)-1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (8p) Yield: 89%, m.p: 154.3-156.6°C, ^{1}H NMR ($CDCl_3$): δ 6.84-6.27 (m, 5H), 5.13 (d, *J* = 5.64 Hz, 1H), 4.43 (dd, *J* = 6.18, 9.17 Hz, 1H), 4.20-4.11 (m, 5H), 3.87 (s, 3H), 3.64 (s, 3H), 2.97-2.64 (m, 4H), IR ν_{max} cm^{-1} 2910, 1760, 1450, 1250.

Cytotoxic assay

Cytotoxicity was measured by the sulforhodamine B (SRB) method (Skehan *et al.,* 1990). Viable cells were seeded in the growth medium (180 μL) into 96 well

microtiter plates ($3\text{-}4\times10^4$ cells per each well) and allowed to attach overnight. The test samples were dissolved in DMSO and adjusted for the final sample concentrations ranging from 0.3 μg/ml to 30 μg/ml by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to < 0.1%.

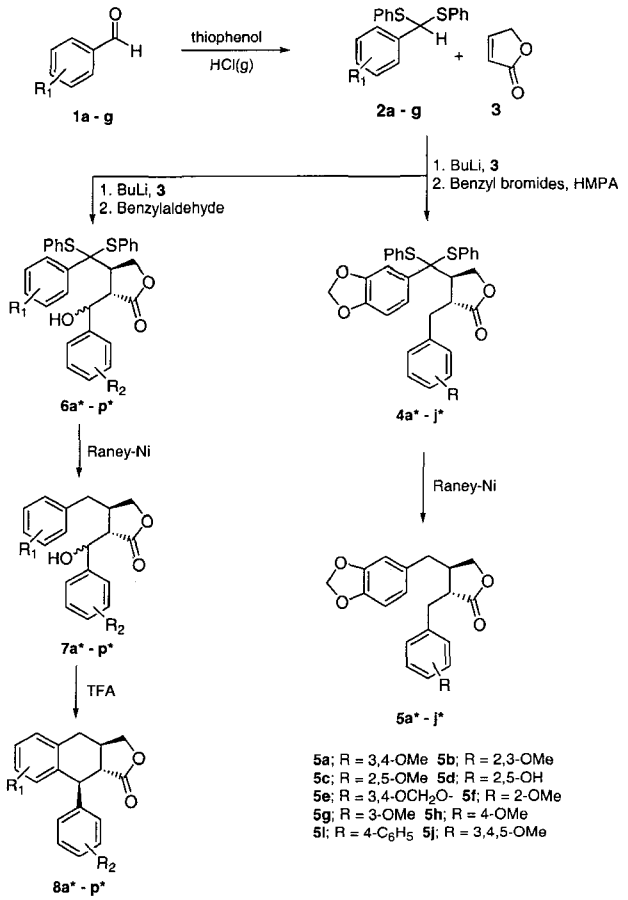
After 72 hrs incubation, the medium was removed and the remaining cells were fixed using 10% trichloroacetic acid (TCA) for 1 hr at 4°C. The TCA-treated cells were washed extensively with water and dried in air. Subsequently, 50 μL of SRB solution (0.4% in acetic acid) was added to each well at room temperature. After standing for 1 hr, the wells were washed 3~4 times with 1 % acetic acid and dried in air. The bound dye was dissolved in Tris base (100 μL of 10 mM). The absorbance of the Tris solution was measured using a micro-plate reader at 520 nm. The ED_{50} value was defined as the concentration of DPT needed to reduce a 50% of absorbance relative to the vehicle-treated controls.

RESULTS AND DISCUSSION

Chemistry

Racemic *trans*-2,3-dibenzylbutyrolactones were synthesized by conjugate addition of dithioacetal anions to 2-buten-4-olide followed by trapping of the enolate anion so generated with the appropriate substituted benzyl bromide, using the original synthetic strategy of Ziegler (Ziegler *et al.,* 1978). The method is characterized by the fact that racemic *trans*-2,3-configurated compounds are obtained (Scheme 1). Aryl dithianes **2a-2g** were prepared by the reaction of the corresponding aromatic aldehydes with thiophenol and dry HCl (Andrew *et al.,* 1988). Treatment of the corresponding benzyl alcohols with triphenylphosphine and bromine in acetonitrile afforded benzyl bromides in 55%-80% yield (Eckart *et al.,* 1996). Dibenzylbutyrolactone dithioacetals **4a-4j** were prepared from dithioacetal **2a** of 3,4-methylenedioxybenzaldehyde by reacting butyllithium and 2-butenolide in THF at -78°C followed by direct alkylation with the benzyl bromides in the presence of hexamethylenephosphoramide (HMPA). Desulphrization of **4a-4j** was achieved by treatment with Raney-Ni in refluxing ethanol and afforded *trans*-2,3-dibenzylbutyrolactones, **5a-5j** in 13%-25% overall yield.

Next, 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (TNL) derivatives were prepared from various dithioacetals by a similar method of *trans*-2,3-dibenzylbutyrolactones (Scheme 1). α-Hydroxy dibenzylbutyrolactone dithioacetals **6a-6p** were prepared from dithioacetals by reacting butyllithium and 2-buten-4-olide in THF at -78°C followed by direct addition with benzaldehydes. Desulphrization were achieved



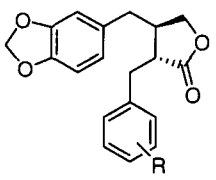
5a; R = 3,4-OMe **5b**; R = 2,3-OMe
5c; R = 2,5-OMe **5d**; R = 2,5-OH
5e; R = 3,4-OCH_2O- **5f**; R = 2-OMe
5g; R = 3-OMe **5h**; R = 4-OMe
5i; R = 4-C_6H_5 **5j**; R = 3,4,5-OMe

8a; R_1 = 3,4-OCH_2O-, R_2 = 3,4-OMe **8b**; R_1 = 3,4-OCH_2O-, R_2 = 2,3-OMe
8c; R_1 = 3,4-OCH_2O-, R_2 = 2,5-OMe **8d**; R_1 = 3,4-OCH_2O-, R_2 = 2,5-Me
8e; R_1 = 3,4-OCH_2O-, R_2 = 5-Br-2-OMe **8f**; $R_1 = R_2$ = 3,4-OCH_2O-
8g; R_1 = 3,4-OCH_2O-, R_2 = 2-OMe **8h**; R_1 = 3,4-OCH_2O-, R_2 = 4-OMe
8i; R_1 = 3,4-OCH_2O-, R_2 = 4-C_6H_5 **8j**; R_1 = 3,4-OCH_2O-, R_2 = 3,4,5-OMe
8k; R_1 = 3-OMe, R_2 = 2,5-OMe **8l**; R_1 = 2,3-OMe, R_2 = 2,5-OMe
8m; R_1 = 3,4-OMe, R_2 = 2,5-OMe **8n**; R_1 = 3,4,5-OMe, R_2 = 2,5-OMe
8o; R_1 = 2,4-OMe, R_2 = 2,5-OMe **8p**; R_1 = 3,4-OCH_2CH_2O-, R_2 = 2,5-OMe

Scheme 1. Synthesis of 2,3-dibenzylbutyrolactones and 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactones. An asterick indicates 2,3,4- or 2,3-*trans* form

by a same method as mentioned above and afforded α-hydroxy dibenzylbutyrolactones. Electrophilic ring closure of **7a-7p** with TFA leads to the "trans" product of TNL ((±) deoxyisopodophyllotoxin) derivatives in 45%-55% yield. The ^{1}H-NMR spectrum of TNL derivatives was complex. Thus, the coupling constant between H-2 and H-3 of the derivatives could not be measured. But it was assumed by analogy with previous work (Ziegler *et al.,* 1978; Andrew *et al.,* 1983) that the addition had taken place in such a way as to give the *trans* configuration of two benzyl groups on the lactone ring. Moreover, the *trans* configuration of C3-C4 was assigned on the basis of the observed coupling constant (10.7 Hz) between H-3 and H-4. From these results, it could be concluded that the configuration of C3, C4, and C5 of TNL derivatives is *trans*.

Table 1. Cytotoxic activity of yatein derivatives against some cancer cell lines



No. of compd.	R	Cytotoxicity[a] (ED_{50}; μM)[b]	
		A549	SK-MEL-2
5a	3,4-Dimethoxy	>50	>50
5b	2,3-Dimethoxy	>50	>50
5c	2,5-Dimethoxy	49	>50
5d	2,5-Dihydroxy	>50	>50
5e	3,4-Methylenedioxy	>50	>50
5f	2-Methoxy	>50	>50
5g	3-Methoxy	>50	>50
5h	4-Methoxy	>50	>50
5i	4-Biphenyl	>50	>50
5j	3,4,5-Trimethoxy	>50	>50
(-) Yatein		1.1	1.4
DPT		0.053	0.011

[a]A549, human lung carcinoma; SK-MEL-2, human melanoma. [b]Drug concentration required to inhibit the growth of cancer cells by 50%.

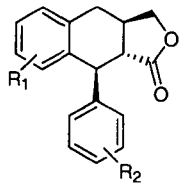
Cytotoxicity

Cytotoxic activity of trans-2,3-dibenzylbutyrolactone (**5a-j**) was evaluated against A549 and SK-MEL-2 cells, and the result was demonstrated in Table 1. All compounds tested, which belong to a series of racemic yatein were nearly inactive against all of the assayed cell lines (ED_{50}; > 40 μM).

Previously, it was reported that (-) yatein (AC; 1.25 μM) isolated from *Juniferus chinenesis* showed potent cytotoxic activity (Novelo *et al.*, 1993). On the other hand, racemic yatein tested (**5j**) was nearly inactive. From these results, it could be concluded that the cytotoxic action of the yatein series is stereospecific, suggesting that (+) yatein behaves as an antagonist to the active (-) yatein on a receptor binding site, although its mode of action was not fully understood.

Next, 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (TNL) derivatives were evaluated for the cytotoxic activity. The data was demonstrated in Table 2. For comparison purpose, the average ED_{50} values (AC) of the compounds in three cancer cell lines were calculated and the results are included in Table 2. Contrary to the above compounds,

Table 2. Cytotoxic activity of (±) 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone derivatives against some cancer cell lines



No. of comp	R_1	R_2	Cytotoxicity[a] (ED_{50}; μM)[b]			
			A549	SK-MEL-2	MCF7	AC[c]
8a	$-OCH_2O-$	3,4-dimethoxy	7.3	8.1	6.6	7.33
8b	″	2,3-dimethoxy	8.6	11.4	7.6	9.20
8c	″	2,5-dimethoxy	0.9	0.7	0.6	0.73
8d	″	2,5-dimethyl	11.4	11.2	12.3	11.6
8e	″	5-bromo-2-methoxy	9.9	10.2	11.2	10.4
8f	″	3,4-methylenedioxy	11.9	12.2	13.2	12.4
8g	″	2-methoxy	7.9	9.2	8.9	8.67
8h	″	4-methoxy	>30	>30	>30	>30
8i	″	4-biphenyl	>30	>30	>30	>30
8j	″	3,4,5-trimethoxy	0.79	0.60	0.71	0.72
8k	2-methoxy	2,5-dimethoxy	>30	>30	>30	>30
8l	2,3-dimethoxy	″	>30	>30	>30	>30
8m	3,4-dimethoxy	″	5.3	4.8	5.5	5.20
8n	3,4,5-trimethoxy	″	>30	>30	>30	>30
8o	2,5-dimethoxy	″	>30	>30	>30	>30
8p	$-OCH_2CH_2O-$	″	1.7	1.5	1.1	1.43
DPT			0.028	0.012	0.02	0.02
VP16			2.34			2.34

[a]A549, human lung carcinoma; SK-MEL-2, human melanoma; MCF-7, human breast carcinoma. [b]Drug concentration required to inhibit the growth of cancer cells by 50%. [c]AC; average of ED_{50} value.

many of this series of compounds tested showed a considerable cytotoxic activity against all of the cell lines assayed. Despite analogue **8j** having the same ring A, C structure as (-) deoxypodophyllotoxin (DPT) showed the strongest activity among the compounds listed in Table 2, the **8j** was more 35 times less cytotoxic than the natural (-) DPT. Previously, it was reported that (±) deoxyisopodophyllotoxin (isoDPT) with all 2,3,4-*trans* configuration significantly inhibited tubulin polymerization, while (-) isoDPT with 2R, 3S and 4R configuration was nearly inactive, indicating that absolute configuration of 2,3,4-position in DPT derivatives plays an important role for the activity (Zavala *et al.,* 1980).

Cytotoxic activity of *trans*-2,3-dibenzylbutyrolactone and TNL derivatives, connected by a butenolide moiety, was observed to depend upon 2,3-configuration of the butenolide moiety.

As for the substituent effect of the ring C, the cytotoxic activity of subseries **8a-8j** with fixed 3,4-methylenedioxy group was compared, resulting in decrease of the average ED_{50} values in the order of **8c** > **8j** > **8a** ≅ **8b** ≅ **8f** ≅ **8g** > **8h** ≅ **8i**. It indicates that presence of a para-methoxy group shows much higher ED_{50} values than otherwise substituted pattern (**8h** *vs.* **8g**). Considering a negligible electronic effect, a steric effect seems to be important for the activity. 2-Methoxy group should enlarge the angle between the butenolide moiety and the ring C so that an optimal angle ensues for the receptor binding. The angle dependence of DPT, diphenyloxazolone, and combretoxazolones were discussed elsewhere (Kim *et al.,* 2002). Analogue **8c** having a 2,5-dimethoxyphenyl unit showed about the same cytotxic activity as **8j**, while otherwise substituted compounds in this subseries of compounds show less activity.

As mentioned above, 2,5-substitution in ring C largely enhanced the cytotoxic activity. So we synthesized some compounds with 2,5-dimethoxy group in ring C and various groups in ring A as a second subseries (**8k-8p**). This subseries was found to show much less cytotoxic activity than the first group (**8a-8j**). From this observation, we found that presence of 3,4-methylenedioxy group in ring A of TNL derivatives plays a key role for the cytotoxic activity.

Among others, **8m** and **8p** with a 3,4-dimethoxyphenyl group and 3,4-ethylenedioxyphenyl in ring A, respectively, displayed the potent activity, while other ones were nearly inactive (ED_{50}, > 30 μM). The chemical similarity of 3,4-ethylenedioxy and 3,4-dimethoxy group with the methylenedioxy group seems to be important for the activity.

With the aim of investigating the cause of diminished activity of 2,3-dibenzylbutyrolactone analogues in comparison with that of the DPT derivatives, we performed molecular modeling studies using Sybyl® (6.6 version) software. We calculated the distance between C-4 in ring A and C'-2 in ring C of **8j** and (±) yatein **5j** by conformational analysis. The distance between C-4 and C'-2 of **5j** (8.987Å) was 3 times longer than that of **8j** (3.181Å, 0.72 μM), which showed a stronger cytotoxic activity than the former one (**5j**; AC, > 50 μM). For this reason, it is concluded that the difference in cytotoxicity comes from difference in distance between the two aromatic rings of (±) 2,3-dibenzylbutyrolactones and (±) TNL derivatives, shorter the distance bringing stronger activity.

CONCLUSION

The cytotoxic activity was dependent on distance between the ring A and C of dibenzyl-γ-butyrolactone and 1,2,3,4-tetrahydro-2-naphthoic acid γ-lactone (TNL) derivatives. The latter compounds with a shorter distance between C-4 in ring A and C'-2 in ring C showed a stronger cytotoxic activity than the former ones. In TNL series, presence of 3,4-dioxy group in ring A and 2-methoxy group in ring C was essential for the enhancement of the activity. Since the synthetic racemic yatein showed no activity, it is necessary to resolve the racemate and test the cytotoxic activity of the isolated enantiomers for elucidating their stereospecifity and the activity relationship.

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