



## Anticancer Activity of 1-Phenylnaphthalene and Pericarboxyl Lignans

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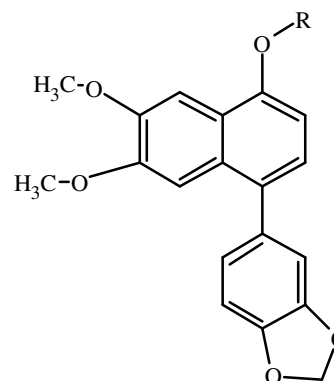
1-Phenyl naphthoic acid, its methyl ester and pericarboxyl lignan lactone system were synthesized and studied for their anticancer activity using MTT assay. The results were found to be significant, causing the inhibition of MCF-7 cells by tumor necrosis factor alpha (TNF- $\alpha$ ) and showed a dose dependent inhibition of MCF-7 cells.

**Key Words:** 1-Phenylnaphthalene, Pericarboxyl lactone, MTT assay.

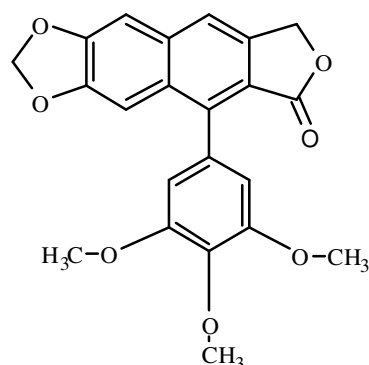
### INTRODUCTION

A cancer cell<sup>1</sup> is a mutant cell that differs little from a normal cell. One difference is rapid growth thus, cancer cell DNA is one target of treatment. The DNA of the rapidly multiplying cells is more exposed than the normal cells. Topoisomerases are enzymes that play an important role in DNA replication. When DNA is ready to multiply, its two strands uncoil and pull apart. Along each strands, a new strand forms to create two copies of the original. Anticancer drugs that are DNA topoisomerase inhibitors block the ability of DNA to uncoil and thus prevent its replication. Lignans have gained importance since their discovery in 1936 because of the numerous biological properties that they possess. Amongst the most important of these properties is the ability of some lignans to arrest the rapid proliferation of cancer cells. Although the aryltetralins are the best represented class of lignans with antitumor activity, members of other subgroups are also known to have antitumor activity<sup>2-7</sup>. Examples of other subclasses of lignans exhibiting antitumor properties are given in **Scheme-I**. Researchers found that the alcoholic extracts of two closely related *Podophyllum* plant species containing lignans exhibited destructive effects towards cancerous cell growths in animals<sup>8a</sup>. The group of lignans derived from the two *Podophyllum* plant species that are responsible for the antitumor activity of the extracts have been identified and have the aryltetralin general structure. The podophyllotoxin was found to be extremely toxic to healthy cells, therefore two semi-synthetic derivatives of podophyllotoxin, Teniposide<sup>TM</sup> and Etoposide<sup>TM</sup>, were developed to overcome the problem. The endeavor was successful and the derivatives are currently

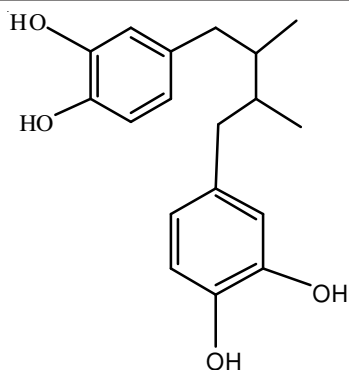
used for the clinical treatment of several varieties of leukemia, lymphoma, small cell lung cancer and germinal testicular cancer<sup>8b</sup>.



1) Diphyllin: R=H; 2) Justicidin A: R=CH<sub>3</sub>



3) Dehydroanhydrocicropodophyllotoxin



4) Nordihydroguaiaretic acid  
Scheme-I

## EXPERIMENTAL

In the present work, we have studied the anticancer activity of 1-phenylnaphthoic acid and pericarboxyl lignan lactones which we have synthesized earlier in our laboratory<sup>9</sup>. The *in vitro* cytotoxic activity of 1-phenylnaphthoic acid and pericarboxyl lactones was studied using MTT assay<sup>10</sup>.

***In vitro* cytotoxic activity of 1-phenylnaphthoic acid and pericarboxyl lactones:** MTT<sup>10</sup> (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. Briefly the compounds were dissolved in DMSO and serially diluted with complete medium to get a range of test concentrations. DMSO concentration was kept < 0.1 % in all the samples. Cell lines maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5 % CO<sub>2</sub> for 96 h. MTT reagent was added to the wells and incubated for 4 h; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550 nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC<sub>50</sub> values for compounds A, B, C and D (Table-1).

## RESULTS AND DISCUSSION

The synthetic compounds (1-phenylnaphthalene and pericarboxyl lactone) inhibited the growth of MCF-7 cells by tumor necrosis factor alpha (TNF- $\alpha$ ) and showed a dose dependent inhibition of MCF-7 cells. Table-1 shows the concentrations required for sample A, B, C and D for 50 % inhibition of MCF-7 cells.

Lignans are of considerable pharmacological and clinical interest in the treatment of cancer and other diseases<sup>11</sup>. After a survey on plants containing lignan, the plants *Phyllanthus amarus* and *Jatropha gossypifolia* were selected which showed structural similarity with the synthesised compounds (1-phenyl naphthalene system). These plants with lignans have potent anticancer activity which has already been proved by various

TABLE-1

Sample code	Sample name	IC <sub>50</sub> (µg/mL)
A	1-Phenylnaphthalene-3-carboxylic acid	40
B	1-Phenyl-6,7-methylenedioxy naphthalene-3-carboxylic acid	20
C	1-Phenyl-6,7-methylenedioxy naphthalene-3-pericarboxyl lactone	30
D	1-Phenyl-6,7,8-trimethoxy naphthalene-3-pericarboxyl lactone	25

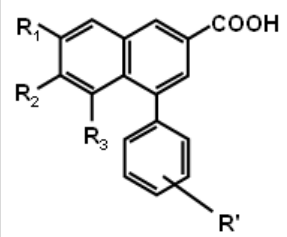
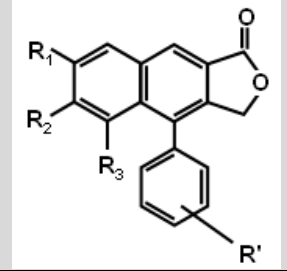
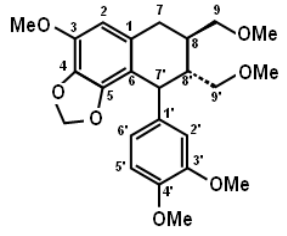
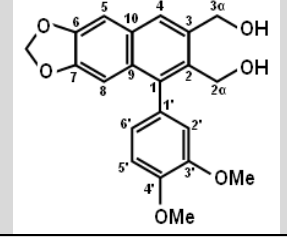
TABLE-2

### REPORT-CYTOTOXICITY ANALYSIS OF SYNTHESIZED COMPOUNDS AGAINST MCF-7

Assay	MTT
Time of incubation	96h
Cell Line	MCF-7
Organism	Homo sapiens (human)
Organ	Mammary gland; breast
Tissue	Epithelium
Disease	Adenocarcinoma
Derived from metastatic site	Pleural effusion
Receptors	Estrogen receptor, expressed
DNA Profile (STR)	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15
Cytogenetic analysis	Modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1 %. There were 29-34 marker chromosomes per S metaphase; 24-28 markers occurred in at least 30 % of cells and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80 % of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Isoenzymes	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1
Age	69 years adult
Gender	Female
Comments	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol <i>via</i> cytoplasmic estrogen receptors and the capability of forming domes. The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with antiestrogens.

researchers<sup>12,13</sup>. *Phyllanthus amarus* extract was found to have significant activity against chemically induced tumor. The

TABLE-3  
ANTICANCER ACTIVITY OF SYNTHESIZED AND NATURAL LIGNANS

Lignans		Structure	Anticancer activity IC <sub>50</sub> values
Synthesized compounds	(A) 1-Phenylnaphthalene-3-carboxylic acid	a and b 	Concentration required for 50 % inhibition of MCF-7 cells A = 40 µg/mL B = 20 µg/mL C = 30 µg/mL D = 25 µg/mL
	(B) 1-Phenyl-6,7-methylenedioxy naphthalene-3carboxylic acid	c and d 	
	(C) 1-Phenyl 6,7-methylenedioxy naphthalene-3-pericarbonyl lactone		
	(D) 1-Phenyl 6,7,8-trimethoxy naphthalene-3-pericarbonyl lactone		
Phyllanthus amarus	Hypophyllanthin lignan		Concentration required for 50 % inhibition of aniline hydroxylase = 540 µg/mL
Jatropha gossypifolia	Arylnaphthalene lignan		Exhibits anticancer activity

Substitutions: (A) R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = H, R' = H, (B) R<sub>1</sub> = R<sub>2</sub> = O-CH<sub>2</sub>-O, R<sub>3</sub> = H, R'=H, (C) R<sub>1</sub> = R<sub>2</sub> = O-CH<sub>2</sub>-O, R<sub>3</sub> = H, R'=H, (D) R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = OCH<sub>3</sub>, R'=H.

extract was also found to inhibit P450 enzymes, which are needed in the activation of carcinogens. This was partially demonstrated by the aniline hydroxylase inhibition data. In a follow-up study the lignans synthesized in our laboratory were also tested for its *in vitro* cytotoxic activity using MTT assay, the results were found to be significant were 1-Phenyl naphthalene system and pericarbonyl lignans inhibited the growth of MCF-7 cells by tumor necrosis factor alpha (TNF- $\alpha$ ) and showed a dose dependent inhibition of MCF-7 cells, depicted in Table-2. Table-3 is plotted for anticancer activity of synthesized and natural lignans with 1-phenylnaphthalene system. Thus, the study reveals that the study of naturally occurring and related organic compounds with 1-phenylnaphthalene system possesses significant anticancer activity.

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