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SYNTHESIS AND CHARACTERISATION OF NOVEL ANTIMITOTIC ANALOGUES OF PODOPHYLLOTOXIN

Sadashivamurthy B*, Pragasam Antony*.
PG Department of Chemistry, Maharani's Science College for Women, Mysore, Karnataka, India.
*** Corresponding Author: sadashivamurthyb@gmail.com**

ABSTRACT

Objective: To synthesis novel analogues of podophyllotoxin and their spectrometric characterization

Mathods: Tetralone esters were used as starting material to synthesis hydroxy methylene tetralone esters and hydroxy methylene tetralone acids as an intermediate compound to form tetra-substituted di-hydroxyl derivatives

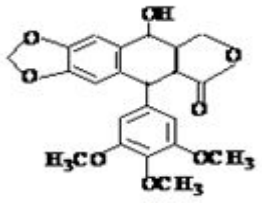
Findings: The tetra-substituted di-hydroxyl derivatives of tetralone esters on dehydrating and cyclisation with p-TsCl/py results podophylloxin analogues 1- Hydroxy-2-hydroxylmethyl-3-carboxy-4-(p-tolyl)-6, 7-dimethoxy - 1,2,3,4-tetrahydronaphthalene(72% yield), 6-methoxy-7-methyl-9-p-tolyl naphtho [2.3-C] furan-1- (3H, 4H, 9H) one(73% yield), 7-Chloro-6-methoxy-9-p-tolyl naphtho [2, 3-C] furan-1- (3H, 4H, 9H) one(72% yield) and 9-Cyclohexyl-6, 7-dimethoxy naphtho- [2,3-C] furan-1- (3H, 4H, 9H) one(70% yield). The structures were confirmed by Proton NMR, IR, Mass spectroscopy and C H analysis.

Novelty: These podophyllotoxin analogues are novel derivatives as these posses strong electron releasing substituents and optimum electron flow in the molecules and their antimitotic activities can be evaluated.

Key words: Podophyllotoxin, analogues, Tetralone esters, p-TsCl/py

1 Introduction

Podophyllotoxin(1) is an antimitotic agent which has been extracted from two important medicinal plants named Podophyllum emodi an Indian species and Podophyllum peltatum a North American species that belong to the family of berbideracea[(1)]. Traditionally, it is used to cure the venereal wart condyloma acuminatum with the topical application of podophyllin in oil [(2)]. This led to the studies of the action of podophyllin on tumor tissues and to intensive chemical examination of the constituents of podophyllin [(3, 4)].



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The structure was confirmed by total synthesis of podophyllotoxin by many shorter synthetic routes and asymmetric synthesis [5, 6]. Synthetic analogous synthesized followed by Jensler's method shown antimitotic activity [7]

Podophyllotoxin and several of its analogues show wide variety of biological activities such as cathartic, cytotoxic, antimitotic, anticancer, antitropical skin disease, antimalarial, virucidal and fungicidal [8-11]. Podophyllotoxin derivatives and vinca alkaloids were the only drugs found markedly inhibit DNA ligases from normal cells [12, 13]. Highly purified Podophyllotoxin (1) efficiently suppress in-vitro and in-vivo immune responses.

Podophyllotoxin is used as the therapeutic agent in the treatment of neoplastic disease is restricted due to the toxic side effects. β-Apopicropodophyllin, a dehydrated isomerised product of podophyllotoxin which has increased antimitotic and structurally modified derivative of podophyllotoxin shown enhanced biological activities [14-18]. The strong antimitotic activity of podophyllotoxin and several of its analogues has led to the investigation of structure activity relationship

Gensler and coworkers synthesized several nonlactone analogues of podophyllotoxin (1). These analogues showed increasing antimitotic activity, but were less active than the parent compound (1). The fact that some of the analogues retained their activity, despite the absence of lactone ring is contradictory to the hypothesis that biological activity involves acylation. The studies of antimitotic activity of analogues of podophyllotoxin(1) or β-apopicropodophyllin shows possibility of increasing the biological activity based molecular modifications like acylation, glycolysis, increasing unsaturation and molecular modeling[19-25]

Hence, the present work was focused to synthesize and characterize the novel podophyllotoxin analogues by replacing trimethoxy phenyl group with p-tolyl and cyclohexyl group, and methylenedioxy group with dimethoxy, methoxy methyl and chloro methoxy group respectively by Gensler's and Chalcone methods.

2 Methodology

2.1 Preparation of hydroxy methylene tetralone esters 2a-d and hydroxy methylene tetralone acids 3a-d

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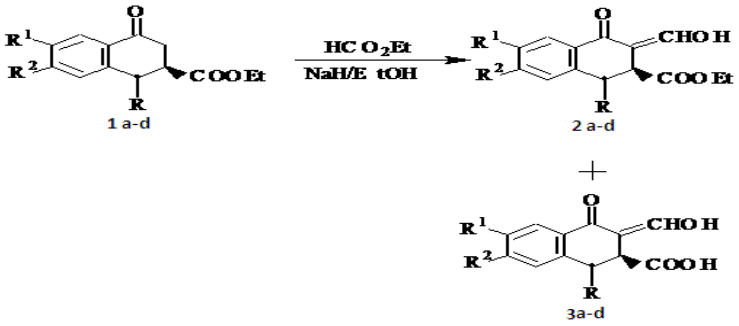

a : $R^1 = R^2 = OCH_3$; R = p-tolyl

b : $R^1 = OCH_3$, $R^2 = CH_3$; R = p-tolyl

c : $R^1 = OH_3$, R^2=Cl; R = p-tolyl

d: R^1=$R^2 = OCH_3$; R = cyclohexyl

Scheme 1

4- (p-Tolyl)-1-oxo-2-hydroxymethylene-3-ethylcarboxy – 6,7- dimethoxy -1,2,3,4 - tetrahydro naphthalene (2a)

Sodium hydride (1.221g, 0.05088mole) was added to absolute ethanol (10ml), dry benzene (150ml) mixture and stirred well for 1h. ethyl formate (10ml) was added drop-wise to the above mixture and stirred for another 1h, followed by drop-wise addition of **1a** (5.1g, 0.01384 mole) in dry benzene (100ml) over a period of 1h. After stirring, the red coloured mixture at room temperature for 12h. it was poured into 2N H_2SO_4 (100ml) in ice (100g). The organic layer separated was washed with water (3x50ml) and extracted into saturated sodium bicarbonate solution (3x50ml), followed by 1% sodium hydroxide solution (3x50ml).

The sodium hydroxide extract was acidified with 2N H_2SO_4 gave yellow solid. It was recrystallised with ethanol gave yellow crystalline solid in 80.65% yield (4.42g), m.p: 99-101^{0}C.

4-(p-Tolyl)-1 oxo-2-hydroxyl methylene-3-ethyl carboxy-7- methoxy -6-methyl-1, 2, 3, 4 - tetrahydronaphthalene (2b)

Prepared from **1b** (5.4g, 0.01532 mole), sodium hydride (1.351g, 0.0563 mole) and ethyl formate (10ml) as a yellow crystalline solid in 77.31% yield (4.58), m.p: 80-82^{0}C.

4-(p-Tolyl)-1-oxo-2-hydroxymethylene-3-ethyl-carboxy-6-chloro - methoxy-1, 2,3,4 - tetrahydronaphthalene (2c)

Prepared from **1c** (5.2g, 0.01394 mole), sodium hydride (1.258g, 0.0524 mole) and ethyl formate (10ml) as pale yellow coloured semisolid in 77.00% yield (4.3g).

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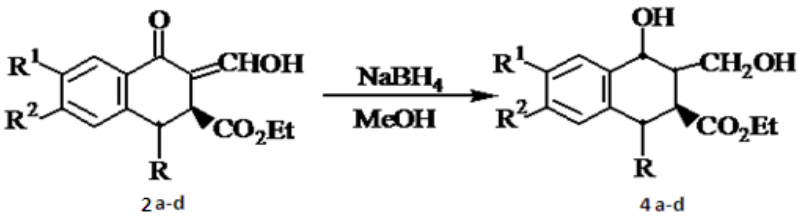
4-Cyclohexyl-1-oxo-2-hydroxylmethylene-3-ethyl-carboxy- 6,7-dimethoxy-1, 2,3,4-tetrahydronaphthalene (2d)

Prepared from **1d** (5.0g, 0.0138 mole), sodium hydride (1.221g, 0.0509 mole) and ethyl formate as pale yellow crystalline solid in 78% yield (4.2g), m.p: 76-78^{0}C.

2.2 Preparation of the dihydroxy esters 4a-d



2a-d 4a-d

a : $R^1 = R^2 = OCH_3$; R = p-tolyl

b : $R^1 = OCH_3$, $R^2 = CH_3$; R = p-tolyl

c : $R^1 = OH_3$, $R^2 = Cl$; R = p-tolyl

d : $R^1 = R^2 = OCH_3$; R = cyclohexyl

Scheme 2

1- hydroxy–2–hydroxy methyl –3-ethyl carboxy-4- (tolyl)-6,7 dimethoxy-1, 2,3,4-tetrahydronaphthalene (4a)

To a solution of **2a** (4g, 0.01008 mole) in absolute methanol (60ml), sodium borohydride (4.2g) in absolute methanol (60ml) was added during 1h at room temperature. At an hourly interval, a solution of sodium borohydride (1g) in methanol (10ml) was added three times. The reaction mixture after stirring at room temperature for 5h was concentrated to 40ml, acidified with 2N HCl and then the pH of the solution was adjusted to 8 by adding 1% aqueous ammonium hydroxide solution. The product was extracted into ether (3x50ml), the ether layer was washed with cold 1% sodium hydroxide solution (2x40ml), water (2x40ml) and then dried over anhydrous Na_2SO_4. On evaporation of the solvent gave pink coloured semisolid in 71.79% yield (2.9g).

1-Hydroxy-2-hydroxymethyl-3-ethyl caroxy-4- (P-tolyl)-7- methoxy-6-methyl-1, 2, 3, 4 -tetrahydronaphthalene (4b)

Prepared from **2b** (4g, 0.0105 mole) and sodium borohydride (4.8g, 0.1268 mole) in methanol (80ml) as pale yellow coloured semisolid in 74.93% (3.02g),

1-Hydroxy-2-hydroxymethyl-3-ethyl carboxy-4- (p-tolyl)-6- chloro-7-methoxy-1, 2, 3, 4 -tetrahydronaphthalene (4c)

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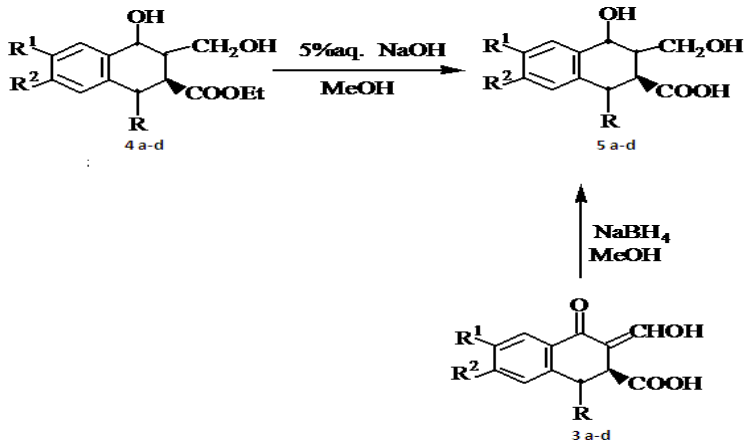
Prepared from **2c** (3.9g, 0.00972 mole) and sodium borohydride (4.8g, 0.1268 mole) in methanol (80ml) as a yellow coloured semisolid in 78.72% yield (3.1g).

1-Hydroxy-2-hydroxylmethyl-3-ethyl carboxy-4-cyclohexyl- 6, 7-dimethoxy-1, 2, 3, 4 tetrahydronaphthalene (4d)

Prepared from **2b** (4.0g, 0.01029 mole) and sodium borohydride (4.8g, 0.1268 mole) in methanol (80ml) as an orange red coloured semisolid in 67.82% yield (2.74g).

2.4 Preparation of dihydroxy acids 5a-d



a : $R^1 = R^2 = OCH_3$; R = p-tolyl

b : $R^1 = OCH_3$, $R^2 = CH_3$; R = p-tolyl

c : $R^1 = OH_3$, $R^2 = Cl$; R = p-tolyl

d: $R^1 = R^2 = OCH_3$; R = cyclohexyl

Scheme 3

1- Hydroxy-2-hydroxylmethyl-3-carboxy-4-(p-tolyl)-6, 7-dimethoxy - 1,2,3,4-tetrahydronaphthalene (5a)

A solution of **4a** (2.5g, 0.00624 mole) in methanol (30ml) and 5% sodium hydroxide (40ml) was refluxed for 3h after removing the methanol under reduced pressure, the alkaline solution was acidified with 2N HCl gave pink coloured solid which on recrystallised with methanol gave pale pink coloured crystalline product in 80% yield (1.83g), m.p. 82-84^{0}C.

1-Hydroxy-2-hydroxyl methyl-3-carboxy-4- (p-tolyl)-7- methoxy-6-methyl-1, 2, 3, 4-tetrahydronaphthalene (5b)

Prepared from **4b** (2.6g, 0.00676 mole) in methanol (30ml) and 5% sodium hydroxide (40 ml) as pale yellow crystalline solid in 76.50% yield (1.84g),

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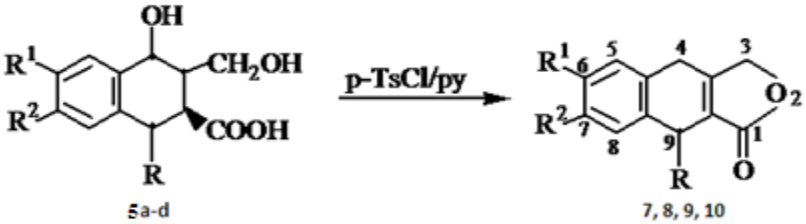
1-Hydroxy-2-hydroxyl methyl-3-carboxy-4- (p–tolyl)-6-chloro - 7-methoxy- 1, 2, 3, 4-tetrahydronaphthalene (5c)

Prepared from **4c** (2.6g, 0.00642 mole) in methanol (30ml) and 5% sodium hydroxide (40 ml) as yellow crystalline solid in 77.31% yield (1.84g), m.p. 91-93^{0}C.

1-Hydroxy-2-hydroxy methyl-3-carboxy-4-cyclohexyl-6, 7- dimethoxy-1, 2, 3, 4 - tetrahydronapthalene (5d)

Prepared from **4d** (1.0g, 0.00254 mole) in methanol (25ml) and 5% sodium hydroxide (30 ml) as pink crystalline solid in 79.74% yield (1.48g), m.p: 77-79^{0}C.

2.4 Preparation of β-apopicropodophyllin analogues 7, 8, 9 and 10



7; a: $R^1 = R^2 = OCH_3$; R = p-tolyl

8; b: $R^1 = OCH_3$, $R^2 = CH_3$; R = p-tolyl

9; c: $R^1 = OH_3$, R^2=Cl; R = p-tolyl

10; d: $R^1 = R^2 = OCH_3$; R = cyclohexyl

Scheme 4

6, 7-dimethoxy-9-p-tolyl naphtha [2, 3-C] furan-1- (3H, 4H, 9H) one (7)

A mixture of **6a** (1.2g, 0.0322 mole), p-toluene sulfonyl chloride (3.5g, 0.0185 mole) and dry pyridine (30ml) in dry benzene (60 ml) was refluxed for 3h. The reaction mixture was cooled to room temperature, washed with 2N HCl (3x50ml) and then with water (2x40ml). The solvent was removed by distillation under reduced pressure gave thick brown residue. The crude product was column chromatographed over silica gel (10mx30) using chloroform as the eluant. The solvent was removed and evacuated at 50^0 C on a rotary evaporator gave orange red coloured crystalline solid in 72.02% yield (0.78g), m.p: 69-71^{0}C.

6-methoxy-7-methyl-9-p-tolyl naphtho [2.3-C] furan-1- (3H, 4H, 9H) one (8)

Prepared from **6b** (1.4g, 0.003928 mole), p-toluene sulfonyl chloride (4.2g, 0.02202mole) and dry pyridine (25ml) in dry benzene (60ml) as orange red coloured crystalline solid in 74.76% yield (0.937g), m.p: 72-74^{0}C.

7-Chloro-6-methoxy-9-p-tolyl naphtho [2, 3-C] furan-1- (3H, 4H, 9H) one (9)

Prepared from **6c** (1.5g, 0.0398 mole), p-toluene sulfonyl chloride (4.3g, 0.002216 mole) and dry pyridine (25ml) in dry benzene (60ml) as light yellow crystalline solid in 71.53% yield (0.978g), m.p: 78-80^{0}C.

9-Cyclohexyl-6, 7-dimethoxy naphtho- [2,3-C] furan-1- (3H, 4H, 9H) one (10)

Prepared from **6d** (0.5g, 0.0137 mole), p-toluene sulfonyl chloride (1.5g, 0.00786 mole) and dry pyridine (15ml) in dry benzene (40ml) as yellow crystalline solid in 70% yield (0.63g), m.p: 75-77^{0}C.

3 Results and Discussion

3.1 Preparative analysis of hydroxy methylene tetralone esters 2a-d and hydroxy methylene tetralone acids 3a-d ((Scheme 1)

4-(p-Tolyl)-1-oxo-2-hydroxymethylene-3-ethylcarboxy–6,7-dimethoxy-1,2,3,4 tetra-hydro naphthalene (2a).

IR (KBr): 3450-3200 (OH), 1740 (ester C=O), 1690 (tetralone C=O), 1625 (conjugated C=C), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ4.0 (s, 6H, OCH_3), δ 4.2 (q, J=7Hz, 2H, ester CH_2), δ 2.3 (s, 3H, CH_3), δ 7.0-7.2 (bm, 6H, Ar-H) δ 5.7 (bs, 1H, vinyl OH), δ 8.3 (s, 1H, vinylic), δ 3.4 (d, J=6.5Hz, 1H, C_4-H), δ 2.9 (d, J=6.8 Hz, 1H, C_3-H), δ 1.2-1.4 (t, 8Hz, 3H, ester CH_3);
Anal. Calcd: For $C_{23}H_{24}O_6$; C, 74.99; H, 6.10%;
Found: C, 74.9; H, 6.08%.
The bicarbonate extract was acidified with 2N H_2SO_4 gave yellow solid. It was recrystallised with ethanol gave yellow crystalline solid in 3.9% yield (0.2g), m.p: 96-98^{0}C.
IR (KBr): 1720-1740 (C=O of COOH), 1690-1710 (C=O of tetralone) cm-^{1};
PMR ($CDCl_3$): δ 9.7-9.9 (bs, 1H, COOH) and no ester group signal.

4-(p-Tolyl)-1 oxo-2-hydroxyl methylene-3-ethyl carboxy-7- methoxy-6-methyl-1, 2, 3, 4 -tetrahydronaphthalene (2b)

IR (KBr): 3400-3200 (OH), 1730 (ester C=O), 1690 (tetralone C=O), 1630 (conjugated C=C), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 7.1-7.2 (bm, 6H, Ar-H), δ 2.5(s, 6H, CH_3), δ 3.9-4.2 (bm, 5H, OCH_3& ester CH_2), δ 5.6 (bs, 1H,vinyl OH), δ 8.3 (s, 1H, vinylic), δ 3.4 (d, J=7Hz, 1H, C_4-H), δ 3.0 (d, J=6.5Hz, 1H, C_3-H), δ 1.1-1.4 (t, 7.8Hz, 3H, ester CH_3);
Anal. Calcd: For $C_{23}H_{24}O_5$; C, 72.61; H, 6.35%;
Found: C, 72.57; H, 6.31%.
The bicarbonate extract was acidified with 2N H_2SO_4 gave a yellow crystalline solid 2b in 3.35% yield (0.18g), m.p: 91-93^{0}C.

PMR ($CDCl_3$): δ 9.7-9.8 (bs, 1H, COOH) and no ester group signal.
Anal. Calcd: For $C_{21}H_{20}O_5$; C, 71.5; H, 5.72;
Found: C, 71.3; H, 5.71%.

4-(p-Tolyl)-1-oxo-2-hydroxymethylene-3-ethyl-carboxy-6-chloro-methoxy-1,2,3,4 tetra hydro-naphthalene (2c)

IR (Nujol): 3400-3200 (OH), 1740 (ester C=O), 1690 (tetralone C=O), 1625 (conjugated C=C), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$):δ 3.9-4.1 (bm, 5H, OCH_3& ester CH_2), δ 2.4 (s, 3H,CH_3) δ 5.7 (bs, 1H, vinylic OH), δ 8.4 (s, 1H, vinylic), δ 3.4 (d, J=8Hz, 1H, C_4-H), δ 2.9 (t, 6.5Hz, 1H, C_3-H), δ 1.2-1.4 (t, 3H, 6Hz, ester CH_3), δ 7.2-7.4 (bm, 6H, Ar-H)
Anal. Calcd: For $C_{22}H_{21}O_5Cl$; C, 65.91; H, 5.28%;
Found: C, 65.86; H, 5.24%.
The bicarbonate extract was acidified with 2N H_2SO_4 gave yellow crystalline solid in 4% yield (0.20g), m.p: 78-80^{0}C;
PMR ($CDCl_3$):δ 9.6-9.8 (bs, 1H, COOH) and no ester group signal;
Anal. Cald: For $C_{20}H_{17}O_5Cl$: C, 64.43; H, 4.59%;
Found: C, 64.40; H, 4.57%.

4-Cyclohexyl-1-oxo-2-hydroxylmethylene-3-ethyl-carboxy- 6,7-dimethoxy-1, 2,3,4-tetrahydronaphthalene (2d)

IR (KBr): 3500-3200 (OH), 1735 (ester C=O), 1680 (tetralone C=O), 1620 (conjugated C=C), 1580 cm^{-1} (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 3.9 (s, 6H, OCH_3), δ 4.2 (q, J=7Hz, 2H, ester CH_2), δ 0.9-2.0 (bm, J=14Hz, cyclohexyl & ester CH_3), δ 5.8 (bs, 1H, vinyl OH), δ 8.2 (s, 1H, vinylic H), δ 7.2-7.4. (m, 2H, Ar-H), δ 3.2 (d, J=4Hz, 1H, C_3-H), δ 2.5 (t, J=5Hz, 1Hz C_4-H);
Anal. Calcd: For $C_{22}H_{28}O_6$; C, 68.02; H, 7.26%;
Found: C, 68.00; H, 7.24%.
The bicarbonate extract was acidified with 2N H_2SO_4 gave yellow crystalline solid in 8% yield (0.399g), m.p: 81-83^{0}C.
PMR ($CDCl_3$): δ 9.6-9.8 (bs, 1H, COOH) and no ester group signal;
Anal. Calcd: For $C_{20}H_{24}O_6$: C, 66.65; H, 6.71%;
Found: C, 66.61; H, 6.70%

3.2 Preparative analysis of dihydroxy esters 4a-d (Scheme 2)

1-Hydroxy-2-hydroxylmethyl-3-carboxy-4-(p-tolyl)-6,7-dimethoxy-1,2,3,4-tetrahydro-naphthalene (5a)

IR (Nujol): 3550-3200 (OH), 1730 (ester C=O), 1600 cm^{-1} (aromatic C=C) cm^{-1};

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PMR ($CDCl_3$): δ 3.9 (s, 6H, OCH_3), δ 4.2 (q, J=7Hz, 2H, ester CH_2), δ 1.1-1.3 (t, J=8Hz, 3H, ester CH_3) δ 3.2-3.4 (bm, 2H, C_1-H & C_3-H), δ 7.2-7.4. (bm, 6H, Ar-H), δ 2.1-2.4 (bm, 4H, C_2-H & CH_2OH), δ 3.5-3.6 (d, J=6Hz, 1H, C_4-H);
Anal. Calcd: For $C_{23}H_{28}O_6$: C, 68.98; H, 7.04%;
Found: C, 68.96; H, 7.01%.

1-Hydroxy-2-hydroxymethyl-3-ethyl caroxy-4-(P-tolyl)-7- methoxy-6-methyl-1, 2, 3, 4 tetrahydro-naphthalene (4b)
IR (Nujol): 3500-3200 (OH), 1725 (ester C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 4.0-4.2 (bm, 5H, OCH_3 & ester CH_2), δ 2.5 (s, 6H, CH_3), δ 7.0-7.2 (bm, 6H, Ar-H), δ 2.1-2.4 (bm, 4H, C_2-H & CH_2OH), δ 3.0-3.1 (d, J=6Hz, 1H, C_4-H), δ 3.2-3.3 (bm, 2H, C_1-H & C_3-H), δ 0.9-1.2 (t, 8Hz, ester CH_3);
Anal. Calcd: For $C_{23}H_{28}O_5$; C, 71.89; H, 7.33%;
Found: C, 71.88; H, 7.29%.

1-Hydroxy-2-hydroxymethyl-3-ethyl carboxy-4- (p-tolyl)-6- chloro-7-methoxy-1, 2, 3, 4 -tetrahydronaphthalene (4c)
IR (KBr): 3500-3200 (OH), 1725 (ester C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 4.0-4.2 (bm, 5H, OCH_3 & ester CH_2), δ 2.4 (s, 6H, CH_3), δ 7.2-7.4 (bm, 6H, Ar-H), δ 1.3-1.4 (t, J=9Hz, ester CH_3), δ 3.4-3.6 (bm, 2H, C_1-H & C_3-H), δ 2.1-2.4 (bm, 4H, C_2-H & CH_2OH), δ 3.2-3.3 (d, J=6Hz, 1H, C_4-H);
Anal. Calcd: For $C_{22}H_{25}O_5Cl$; C, 65.26; H, 6.96%;
Found: C, 65.24; H, 6.93%.

1-Hydroxy-2-hydroxylmethyl-3-ethyl carboxy-4-cyclohexyl-6,7-dimethoxy-1,2,3,4 tetra hydronaphthalene (4d)
IR (Nujol): 3600-3200 (OH), 1730 (ester C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 3.9-4.2 (bm, 8H, OCH_3 & ester CH_2), δ 0.9-2.0 (bm, 14H, cyclohexyl & ester CH_3), δ 7.0-7.2 (bm, 2H, Ar-H), δ 2.1-2.4 (bm, 4H, C_2-H & CH_2OH), δ 3.3-3.5 (bm, 2H, C_1-H & C_3-H), δ 2.6 (t, J=5Hz, 1H, C_4-H);
Anal. Calcd. For $C_{22}H_{32}O$; C, 67.32; H, 8.21%;
Found: C, 67.30; H, 8.19%

3.3 Preparative analysis of dihydroxy acids 5a-d (Scheme 3)

1-Hydroxy-2-hydroxylmethyl-3-carboxy-4-(p-tolyl)-6,7-dimethoxy-1,2,3,4-tetrahydro-naphthalene (5a)
IR (KBr): 3500-3200 (OH), 1710 (carbonyl (C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 9.4-9.6 (bs, 1H, COOH);
Anal. Calcd: For $C_{21}H_{24}O_6$; C, 67.72; H, 6.49%;
Found: C, 67.69; H, 6.48%.

1-Hydroxy-2-hydroxyl methyl-3-carboxy-4-(p-tolyl)-7-methoxy-6-methyl- 1,2,3,4-tetra hydronaphthalene (5b)
IR (KBr): 3500-3200 (OH), 1710 (carboxyl C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 9.3-9.5 (s, 1H, COOH);
Anal. Calcd: For $C_{21}H_{24}O_5$; C, 70.76; H, 6.78%;
Found: C, 70.69; H, 6.76%.

3.3.3 1-Hydroxy-2-hydroxyl methyl-3-carboxy-4-(p–tolyl)-6-chloro -7-methoxy-1,2,3,4-tetra hydronaphthalene (5c)
IR (KBr): 3500-3250 (OH), 1710 (carboxyl C=O), 1590 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 9.6-9.7 (s, 1H, COOH);
Anal. Calcd: For $C_{20}H_{21}O_5Cl$: C, 63.74; H, 5.6%;
Found: C, 63.71; H, 5.59%.

1-Hydroxy-2-hydroxy methyl-3-carboxy-4-cyclohexyl-6,7-dimethoxy-1,2,3,4- tetrahydro-napthalene (5d)
IR (KBr): 3500-3200 (OH), 1710 (carboxyl C=O), 1580 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$): δ 9.7-9.8 (s, 1H, COOH);
Anal. Calc: For $C_{20}H_{28}O_6$: C, 65.91; H, 7.74%;
Found: C, 65.84; H, 7.69%.

3.4 Preparative analysis of dihydroxy acids 5a-d (Scheme 4)

6, 7-dimethoxy-9-p-tolyl naphtha [2, 3-C] furan-1- (3H, 4H, 9H) one (7)
IR (KBr): 1775 (lactone C=O), 1670 (shoulder tetra substituted C=C), 1595 (aromatic C=C) cm^{-1};
PMR ($CDCl_3$):δ 3.9-4.1 (s, 6H, OCH_3), δ 3.7 (s, 1H, C_9-H), δ7.2-7.4 (bm, 6H, Ar-H); δ 2.5 (s, 6H, CH_3), δ 3.8 (s, 2H, C_4-H), δ 4.9 (s, 2H, C_3-H);
Mass (m/z, % of abundance): 336 (M^+, 100), 334 (25), 245 (10), 244 (33), 299 (8), 91 (11);
Anal. Calcd: For $C_{21}H_{20}O_4$; C, 74.98; H, 5.99%;
Found: C, 74.96; H, 5.95%.

6-methoxy-7-methyl-9-p-tolyl naphtho [2.3-C] furan-1- (3H, 4H, 9H) one (8)
IR (KBr): 1770 (lactone C=O), 1676 (shoulder tetra substituted C=C), 1590 (aromatic C=C) cm^{-1},
PMR ($CDCl_3$):δ 3.9-4.1 (s, 3H, OCH_3), δ 3.6 (s, 1H, C_9-H), δ 7.1-7.3 (bm, 6H, Ar-H); δ 2.4 (s, 6H, CH_3), δ 3.75 (s, 2H, C_4-H), δ 4.8 (s, 2H, C_3-H);
Mass (m/z, % abundance): 320 (M^+, 100), 318 (23), 299 (11), 228 (36), 213 (7), 91 (14);
Anal. Calcd: For $C_{21}H_{20}O_3$: C, 78.72; H, 6.29%;
Found: C, 78.69; H, 6.18%.

7-Chloro-6-methoxy-9-p-tolyl naphtho [2, 3-C] furan-1- (3H, 4H, 9H) one (9)

IR (KBr): 1770 (lactone C=O), 1687 (shoulder tetra substituted C=C), 1590 (aromatic C=C) cm^{-1};

PMR ($CDCl_3$):δ 4.0-4.2 (s, 3H, OCH_3), δ 3.8 (s, 1H, C_9-H), δ 7.1-7.3 (bm, 6H, Ar-H); δ 2.9-3.2 (m, 4H, C_8-H & C_9-H), δ 2.5 (s, 6H, CH_3), δ 3.85 (s, 2H, C_4-H), δ 4.9 (s, 2H, C_3-H);

Mass (m/z, % abundance): 340 (M^+, 98), 338 (20), 249 (13), 248 (34), 233 (9), 91 (13);

Anal. Calcd: For $C_{20}H_{17}O_3Cl$; C, 70.48; H, 5.99%;

Found: C, 70.46; H, 5.98%.

9-Cyclohexyl-6, 7-dimethoxy naphtho- [2,3-C] furan-1- (3H, 4H, 9H) one (10)

IR (KBr): 1770 (lactone C=O), 1663 (shoulder tetra substituted C=C), 1590 (aromatic C=C) cm^{-1},

PMR ($CDCl_3$): δ 1.0-2.0 (bm, 11H, cyclohexyl), δ 3.6 (d, J=6Hz, 1H, C_9-H), δ 3.8 (s, 2H, C_4-H), δ 4.8 (s, 2H, C_3-H), δ 7.0-7.2 (bm, 2H, Ar-H);

Mass (m/z, % abundance): 328 (M^+, 100), 326 (22), 245 (10), 244 (37), 229 (12), 83 (12);

Anal. Calcd: For $C_{20}H_{24}O_6$; C, 73.14; H, 7.36%;

Found: C, 73.09; H, 7.32%.

The IR spectrum of 1-hydroxy-2-hydroxy methyl-3-ethyl carboxy-4- (p-tolyl)-1, 2, 3,4-tetrahydro naphthalene **7** showed a broad absorption at the region 3500-3200cm^{-1} assigned to OH groups and a sharp absorption at 1730cm^{-1} assigned to ester carbonyl group. Compounds **8,9**, and **10** showed the IR spectra similar to that of **7**

4 Conclusion

Tetralone esters were used as starting material to synthesis hydroxy methylene tetralone esters and hydroxy methylene tetralone acids as an intermediate compound to form tetra-substituted di-hydroxyl derivatives by Gensler's and Chalcone methods. Hydroxy methylene tetralone esters under reduction with methanol 1- hydroxy–2–hydroxy methyl –3-ethyl carboxy-4- (tolyl)-6,7 dimethoxy-1, 2,3,4-tetrahydronaphthalene,1-Hydroxy-2-hydroxymethyl-3-ethyl caroxy-4- (P-tolyl)-7- methoxy-6-methyl-1, 2, 3, 4 –tetrahydronaphthalene, 1-Hydroxy-2-hydroxymethyl-3-ethyl carboxy-4- (p-tolyl)-6- chloro-7-methoxy-1, 2, 3, 4 – tetrahydronaphthalene and 1-Hydroxy-2-hydroxylmethyl-3-ethyl carboxy-4-cyclohexyl- 6, 7-dimethoxy-1, 2, 3, 4 tetrahydronaphthalene. The tetra-substituted di-hydroxyl derivatives of tetralone esters on dehydrating and cyclisation with p-TsCl/py results podophylloxin analogues 1- Hydroxy-2-hydroxylmethyl-3-carboxy-4-(p-tolyl)-6, 7-dimethoxy - 1,2,3,4-tetrahydronaphthalene, 6-methoxy-7-methyl-9-p-tolyl naphtho [2.3-C] furan-1- (3H, 4H, 9H) one, 7-Chloro-6-methoxy-9-p-tolyl naphtho [2, 3-C] furan-1- (3H, 4H, 9H) one and 9-Cyclohexyl-6, 7-dimethoxy naphtho-[2,3-C] furan-1- (3H, 4H, 9H) one. The structures were confirmed by Proton NMR, IR, Mass spectroscopy and analytical calculations. These novel derivatives posses potential electron releasing substituents and increased unsaturation which concludes the enhanced activities of the

new derivatives. Thus, it finds lot of future scope for exploring various analogues and their biological study.

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Conflict of interest
Authors declared no conflict of interest.

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