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Synthesis and study of antimitotic activity of new tetralone esters

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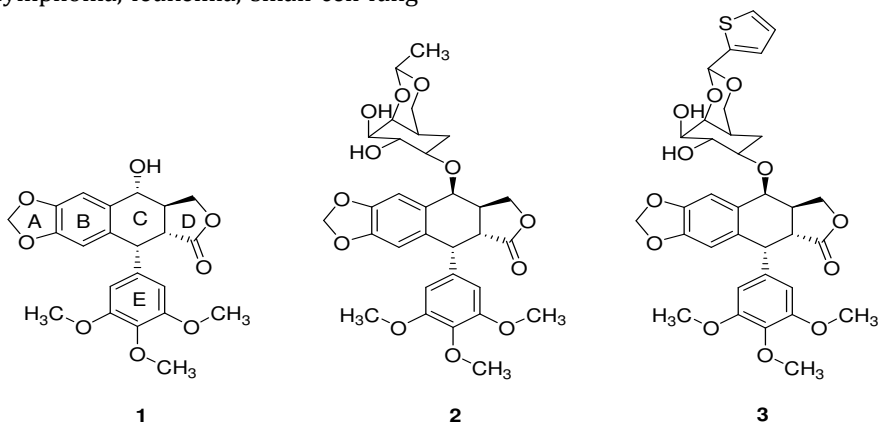
ABSTRACT

Podophyllotoxin is a natural product isolated from two important medicinal plants podophyllum emodi and podophyllum peltatum. Its semi synthetic derivatives etoposide (VP-16) and teniposide (VM-26) are currently in clinical use in the treatment of many cancers, particularly small cell lung carcinoma (SCLC), malignant lymphoma and testicular cancer. In addition podophyllotoxin exhibits strong antimitotic, antiAIDS, antitropical skin disease, antimalarial, virucidal, fungicidal and other biological activities. The new tetralone esters **(9a-d)** of podophyllotoxin analogues are synthesized in good yields by chalcone route to study their structure-activity relationship. All the products obtained are characterized by spectral and elemental analysis data. The antimitotic activities of all the synthesized new tetralone esters are determined by onion-root tip method.

Keywords: Friedel-Crafts acylation, Claisen-Schmidt reaction, Ethyl chloroacetate, Anhyd. Stannic chloride, Tetralone esters, Antimitotic activity.

1. INTRODUCTION

Podophyllotoxin **(1)** is a naturally occurring aryltetralin lignan with important antimitotic [1, 2], antiviral and antineoplastic properties [3]. Podophyllin is a resinous extract of Podophyllum emodi and Podophyllum peltatum belonging to the family of Berberidaceae [4-8]. The use of podophyllotoxin in cancer chemotherapy is restricted due to its toxic side effects and unfavourable solubility. The semi-synthetic derivatives of podophyllotoxin, etoposide **(VP-16, 2)** and teniposide **(VM-26, 3)** are used in the treatment of lymphoma, leukemia, small cell lung cancer, testicular carcinoma, bladder, ovarian, brain cancers and Kaposi's sarcoma [9, 10] (**Figure 1**). Some of its derivatives also exhibit cytotoxic, cathartic and anticancer activities [11-13]. In view of the above reports, it was decided to synthesize the new tetralone esters **(9a-d)** to study their structure-activity relationship by modifying each ring of the podophyllotoxin (A-E). The new tetralone esters obtained were characterized by spectral analysis and evaluated for their antimitotic activities.



1 2 3

Figure - 1: The structure of podophyllotoxin (1) and its semi synthetic derivatives etoposide (2) and teniposide (3).

2. EXPERIMENTAL

2.1. Materials and methods

All the reagents and chemicals of analytical grade were purchased from Merck chemicals and were used without further purification. The melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a FT-IR in KBr disc or Nujol. The ^{1}H NMR spectra were recorded on Jeol 300 MHz and Jeol GSX-400 spectrometer using $CDCl_3$ or DMSO as solvent. The ^{13}C NMR (100 MHz) spectra in DMSO-d_6 solution, was recorded on Bruker DRX-400 instrument at 298 K. The chemical shifts were expressed in δ values relative to the TMS as an internal reference. The mass spectra (ESI-MS) were recorded by Bruker daltonics on ESQUIRE-3000 instrument. The elemental analysis was recorded on a Perkin-Elmer 2400 instrument. The purity of the compounds was checked by TLC on silica gel glass plates in benzene and ethyl acetate mixture (7:0.5). The compounds were purified by column chromatography using silica gel (60-120 mesh) as adsorbent and benzene as eluent.

2.2. Synthesis

2.2.1. General procedure for the synthesis of acetyl Indane (5)

Indane **(4)** (10 g, 0.084 mol) in acetic anhydride (50 ml) containing fused zinc chloride (11.45 g, 0.084 mol) were stirred at room temperature for 12 hrs. After usual workup, the product was obtained and it was recrystallized from ethanol.

2.2.1.1. 5-Acetyl Indane (5)

Color: Yellow solid. Yield: 92.72%. M.p.: 98-99 ^{o}C. IR (KBr, cm^{-1}): ν = 1672 (C=O), 1597 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.08-2.15 (p, 2H, 2-CH_2), 2.58-2.60 (s, 3H, 2-$COCH_3$), 2.93-2.97 (tt, 4H, 1, 3-CH_2), 7.25-7.81 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 199.6, 148.2, 143.6, 134.1, 128.3, 128, 126.1, 33.1, 29.2, 25.1. MS (ESI) m/z: 160.20 (M^+). Anal. Calcd. for $C_{11}H_{12}O$: C, 82.46; H, 7.55. Found: C, 82.42; H, 7.52%.

2.2.2. General procedure for the synthesis of chalcones (7a-d)

5-Acetyl Indane **(5)** (5 g, 0.031 mol) and substituted benzaldehydes **(6a-d)** (0.031 mol) were stirred vigorously in water (40 ml) and ethanol (25 ml) mixture in the presence of sodium hydroxide (1.24 g, 0.031 mol) at 15-30 ^{o}C for 4 hrs. The reaction mixture was kept overnight in an ice bath. The precipitated products were filtered off and recrystallized from ethanol.

2.2.2.1. 1-(2,3-Dihydro-1H-inden-5-yl)-3-(4-methylphenyl)prop-2-ene-1-one (7a)

Colour: Yellow solid. Yield: 95.2%. M.p.: 102-104 ^{o}C. IR (KBr, cm^{-1}): ν = 1669 (C=O), 1593 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.92 (p, 2H, 2-CH_2), 2.31-2.34 (s, 3H, 4-CH_3), 2.73-2.81 (tt, 4H, 1, 3-CH_2), 7.15-7.76 (m, 7H, Ar-H), 7.54 (d, 1H, α-CH), 8.04 (d, 1H, J = 12.0 Hz, β-CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.4, 149.4, 145.0, 144.2, 137.3, 135.1, 132.0, 129.1, 128.7, 128.3, 127.0, 126.2, 121.1, 33.1, 25.2, 24.2. MS (ESI) m/z: 263.4 (M+H). Anal. Calcd. for $C_{19}H_{18}O$: C, 86.99; H, 6.92. Found: C, 86.95; H, 6.90%.

2.2.2.2. 1-(2,3-Dihydro-1H-inden-5-yl)-3-(4-methoxyphenyl)prop-2-ene-1-one (7b)

Colour: Pale yellow solid. Yield: 96.5%. M.p.: 108-110 ^{o}C. IR (KBr, cm^{-1}): ν = 1665 (C=O), 1597 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.94 (p, 2H, 2-CH_2), 2.75-2.80 (tt, 4H, 1, 3-CH_2), 2.81 (s, 3H, 4-OCH_3), 6.91-7.78 (m, 7H, Ar-H), 7.57 (d, 1H, α-CH), 8.03 (d, 1H, J = 12.0 Hz, β-CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.6, 159.2, 149.1, 145.0, 144.2, 134.8, 129.0, 128.6, 127.3, 127.1, 126.8, 121.2, 114.0, 55.7, 32.8, 25.3. MS (ESI) m/z: 279.4 (M+H). Anal. Calcd. for $C_{19}H_{18}O_2$: C, 81.99; H, 6.52. Found: C, 81.96; H, 6.49%.

2.2.2.3. 1-(2,3-Dihydro-1H-inden-5-yl)-3-(4-methylthiophenyl)prop-2-ene-1-one (7c)

Colour: Pale yellow solid. Yield: 92.8%. M.p.: 113-115 ^{o}C. IR (KBr, cm^{-1}): ν = 1661 (C=O), 1591 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.95 (p, 2H, 2-CH_2), 2.52 (s, 3H, 4-SCH_3), 2.76-2.80 (tt, 4H, 1, 3-CH_2), 7.25-7.77 (m, 7H, Ar-H), 7.56 (d, 1H, α-CH), 8.06 (d, 1H, J = 12.0 Hz, β-CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.4, 149.4, 145.1, 144.2, 135.3, 135.0, 131.2, 129.1, 128.6, 127.0, 126.3, 125.8, 121.1, 33.1, 25.1, 14.7. MS (ESI) m/z: 295.16 (M+H). Anal. Calcd. for $C_{19}H_{18}OS$: C, 77.51; H, 6.16. Found: C, 77.49; H, 6.14%.

2.2.2.4. 1-(2,3-Dihydro-1H-inden-5-yl)-3-(3,4-dimethylphenyl)prop-2-ene-1-one (7d)

Colour: Pale yellow solid. Yield: 91.3%. M.p.: 119-121 ^{o}C. IR (KBr, cm^{-1}): ν = 1666 (C=O), 1598 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.09-2.17 (p, 2H, 2-CH_2), 2.30-2.31 (s, 3H, 3,4-CH_3), 2.95-3.00 (tt, 4H, 1, 3-CH_2), 7.16-7.41 (m, 6H, Ar-H), 7.51 (d, 1H, α-CH), 7.74-7.83 (d, 1H, J = 12.0 Hz, β-CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.1, 149.2, 144.8, 144.1, 136.6, 136.0, 135.1, 131.7, 129.2, 128.7, 128.3, 127.1, 126.4, 123.1, 121.2, 29.9, 24.8, 18.1, 17.5. MS (ESI) m/z: 277.1 (M+H). Anal. Calcd. for $C_{20}H_{20}O$: C, 86.92; H, 7.29. Found: C, 86.90; H, 7.28%.

2.2.3. General procedure for the synthesis of cyclopropyl keto esters (8a-d)

Chalcones **(7a-d)** (0.0107 mol), freshly distilled ethyl chloro acetate (1.31 g, 0.0107 mol) and powdered sodium (0.49 g, 0.0214 mol) were stirred in dry benzene (120 ml) at room temperature for 30 hrs. The unreacted sodium and its salts were filtered off. The filtrate was washed with 5% aqueous sodium hydroxide solution (2 X 50 ml), 2% brine solution (2 X 50 ml) and dried over anhyd. Sodium sulphate. The solvent was removed by distillation to give a crude product, which was purified by column chromatography using chloroform as an eluent. The products were recrystallized from ethanol.

2.2.3.1. Ethyl-2-(2,3-dihydro-1H-inden-5-yl-carbonyl)-3-(4-methylphenyl)-cyclopropane-1-carboxylate (8a)

Colour: Brown semi-solid. Yield: 87.5%. IR (KBr, cm^{-1}): ν = 1742 (COO), 1676 (C=O), 1598 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.14-1.29 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.92 (p, 2H, 2-CH_2), 1.94-2.74 (m, 3H, cyclopro-CH), 2.33 (s, 3H, 4-CH_3), 2.78 (tt, 4H, 1, 3-CH_2), 4.15-4.21 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 7.13-7.84 (m, 7H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 192.2, 171.0, 148.1, 143.5, 140.2, 134.3, 133.8, 128.4, 128.1, 127.8, 126.0, 124.7, 61.4, 35.8, 33.4, 33.0, 31.1, 25.1, 24.2, 13.8. MS (ESI) m/z: 348.17 (M^+). Anal. Calcd. for $C_{23}H_{24}O_3$: C, 79.28; H, 6.94. Found: C, 79.26; H, 6.91%.

2.2.3.2. Ethyl-2-(2,3-dihydro-1H-inden-5-yl-carbonyl)-3-(4-methoxyphenyl)-cyclopropane-1-carboxylate (8b)

Colour: Brown solid. M.p.: 109-111 ^{o}C. Yield: 83%. IR (KBr, cm^{-1}): ν = 1745 (COO), 1674 (C=O), 1595 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.11-1.31 (t, 3H, J = 4.0 Hz, $COOCH_2\underline{CH_3}$), 1.91-2.73 (m, 3H, cyclopro-CH), 1.94 (p, 2H, 2-CH_2), 2.80 (tt, 4H, 1, 3-CH_2), 3.81 (s, 3H, 4-OCH_3), 4.17-4.23 (q, 2H, J = 4.0 Hz, $COO\underline{CH_2}CH_3$), 6.89-7.83 (m, 7H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 192.3, 171.4, 157.1, 148.1, 143.6, 135.5, 133.9, 128.2, 127.8, 126.1, 125.7, 113.6, 61.4, 55.8, 40.1, 36.0, 32.8, 25.2, 24.4, 14.1. MS (ESI) m/z: 364.17 (M^+). Anal. Calcd. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.77; H, 6.62%.

2.2.3.3. Ethyl-2-(2,3-dihydro-1H-inden-5-yl-carbonyl)-3-(4-methylthiophenyl)-cyclopropane-1-carboxylate (8c)

Colour: Brown solid. M.p.: 116-118 ^{o}C. Yield: 81.3%. IR (KBr, cm^{-1}): ν = 1741 (COO), 1671 (C=O), 1596 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.18-1.30 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.95 (p, 2H, 2-CH_2), 1.95-2.71 (m, 3H, cyclopro-CH), 2.52 (s, 3H, 4-SCH_3), 2.76 (tt, 4H, 1, 3-CH_2), 4.16-4.22 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 7.19-7.86 (m, 7H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 192.5, 171.1, 148.2, 143.7, 139.7, 134.0, 132.8, 128.3, 128.0, 126.2, 125.1, 61.6, 40.2, 36.1, 33.0, 25.3, 24.6, 14.7, 13.9. MS (ESI) m/z: 380.14 (M^+). Anal. Calcd. for $C_{23}H_{24}O_3S$: C, 72.60; H, 6.36. Found: C, 72.56; H, 6.33%.

2.2.3.4. Ethyl-2-(2,3-dihydro-1H-inden-5-yl-carbonyl)-3-(3,4-dimethylphenyl)-cyclopropane-1-carboxylate (8d)

Colour: Yellow solid. M.p.: 125-127 ^{o}C. Yield: 90.8%. IR (KBr, cm^{-1}): ν = 1744 (COO), 1679 (C=O), 1598 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.21-1.29 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.96 (p, 2H, 2-CH_2), 1.96-2.73 (m, 3H, cyclopro-CH), 2.32 (s, 6H, 3,4-CH_3), 2.80 (tt, 4H, 1, 3-CH_2), 4.18-4.21 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 6.96-7.83 (m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 192.0, 170.8, 148.0, 143.1, 140.2, 135.9, 134.1, 133.0, 128.1, 127.7, 127.4, 126.6, 126.0, 122.0, 61.4, 40.1, 36.4, 33.0, 25.1, 24.2, 17.8, 17.4, 14.0. MS (ESI) m/z: 362.19 (M^+). Anal. Calcd. for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 79.52; H, 7.21%.

2.2.4. General procedure for the synthesis of tetralone esters (9a-d)

A solution of cyclopropyl keto esters **(8a-d)** (0.0082 mol) in dry dichloromethane (75 ml) was added dropwise to a magnetically stirred solution of anhyd.Stannic chloride (2.14 g, 0.0082 mol) and acetic anhydride (1.67 g, 0.0164 mol) in dichloromethane (75 ml) for half an hr. at 0 ^{o}C and further stirred for 6 hrs. After treating the reaction mixture with 5N HCl solution (50 ml), the organic layer was washed with 10% NaOH solution (2 X 50 ml) and finally with water. The crude product was purified by column chromatography using benzene as an eluent.

2.2.4.1. Ethyl 5-oxo-8-(4-methylphenyl)-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]naphthalene-7-carboxylate (9a)

Colour: Reddish brown semi solid. Yield: 79.6%. IR (KBr, cm^{-1}): ν = 1744 (COO), 1697 (C=O), 1594 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.17-1.29 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.94 (p, 2H, 2-CH_2), 2.32 (s, 3H, 4-CH_3), 2.77-3.04 (dd, 2H, 6-CH_2), 2.78 (tt, 4H, 1, 3-CH_2), 3.62 (q, 1H, J = 4.0 Hz, 7-CH), 4.14-4.23 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 4.65 (d, 1H, J = 12.0 Hz, 8-CH), 7.16-7.79 (m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 196.6, 172.7, 148.3, 141.1, 139.8, 137.5, 135.3, 131.2, 129.4, 129.0, 128.3, 127.7, 61.3, 46.2, 41.0, 37.2, 33.1, 32.8, 25.0, 24.1, 13.7. MS (ESI) m/z: 348.17 (M^+). Anal. Calcd. for $C_{23}H_{24}O_3$: C, 79.28; H, 6.94. Found: C, 79.24; H, 6.92%.

2.2.4.2. Ethyl 8-(4-methoxyphenyl)-5-oxo-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]naphthalene-7-carboxylate (9b)

Colour: Reddish brown semi solid. Yield: 85.4%. IR (KBr, cm^{-1}): ν = 1745 (COO), 1699 (C=O), 1593 (C=C). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.22-1.30 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.92 (p, 2H, 2-CH_2), 2.75-3.02 (dd, 2H, 6-CH_2), 2.76 (tt, 4H, 1, 3-CH_2), 3.60 (q, 1H, J = 4.0 Hz, 7-CH), 3.82 (s, 3H, 4-OCH_3), 4.11-4.21 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 4.67 (d, 1H, J = 12.0 Hz, 8-CH), 6.91-7.78 (m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 196.5, 173.0, 157.8, 148.2, 141.0, 137.7, 135.1, 131.1, 129.0, 128.7, 128.2, 114.4, 61.2, 55.4, 46.2, 41.1, 37.5, 33.0, 32.6, 24.9, 13.6. MS (ESI) m/z: 364.17 (M^+). Anal. Calcd. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.77; H, 6.60%.

2.2.4.3. Ethyl 8-(4-methylthiophenyl)-5-oxo-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]naphthalene-7-carboxylate (9c)

Colour: Reddish brown semi solid. Yield: 78.5%. IR (KBr, cm^{-1}): ν = 1742 (COO), 1691 (C=O), 1596 (C=C). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.24-1.31 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.95 (p, 2H, 2-CH_2), 2.51 (s, 3H, 4-SCH_3), 2.74-3.03 (dd, 2H, 6-CH_2), 2.82 (tt, 4H, 1, 3-CH_2), 3.63 (q, 1H, J = 4.0 Hz, 7-CH), 4.14-4.23 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 4.68 (d, 1H, J = 12.0 Hz, 8-CH), 7.16-7.80 (m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 196.1, 172.7, 148.3, 141.0, 139.0, 137.9, 133.5, 131.2, 128.8, 128.3, 128.0, 126.8, 61.3, 46.2, 41.0, 37.4, 33.1, 32.8, 25.0, 14.6, 14.0. MS (ESI) m/z: 380.14 (M^+). Anal. Calcd. for $C_{23}H_{24}O_3S$: C, 72.60; H, 6.36. Found: C, 72.57; H, 6.35%.

2.2.4.4. Ethyl 5-(3,4-dimethylphenyl)-8-oxo-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]naphthalene-6-carboxylate (9d)

Colour: Reddish brown semi solid. Yield: 76.8%. IR (KBr, cm^{-1}): ν = 1746 (COO), 1692 (C=O), 1595 (C=C). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.26-1.29 (t, 3H, J = 4.0 Hz, $COOCH_2CH_3$), 1.96 (p, 2H, 2-CH_2), 2.34 (s, 6H, 3,4-CH_3), 2.76-3.04 (dd, 2H, 6-CH_2), 2.80 (tt, 4H, 1, 3-CH_2), 3.62 (q, 1H, J = 4.0 Hz, 7-CH), 4.12-4.22 (q, 2H, J = 4.0 Hz, $COOCH_2CH_3$), 4.69 (d, 1H, J = 12.0 Hz, 8-CH), 6.98-7.78 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 196.8, 172.9, 148.5, 140.8, 139.3, 138.0, 137.1, 134.1, 131.2, 129.7, 129.2, 128.8, 128.2, 125.0, 61.5, 46.8, 41.1, 37.7, 33.1, 32.5, 25.0, 18.1, 17.6, 13.8. MS (ESI) m/z: 362.19 (M^+). Anal. Calcd. for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 79.52; H, 7.22%.

2.3. Antimitotic activity

The antimitotic activities of synthesized new tetralone esters of podophyllotoxin analogues **(9a-d)** were determined by onion root tip method and the ID_{50} was calculated [14]. The required materials were acetoorcein solution, compound microscope, cover slips, glass slides, hydrochloric acid (0.1 N), Carney's solution II, 70% ethanol and test samples (100, 200 and 400 ppm). To study the effect of new tetralone esters on somatic cells, the onion base was immersed to an extent of about 0.5 cm in a sample tube and control solution tube (ca 7x3 cm). After removing the old roots, onion base immersion was continued to 24 hrs, for germination. Then the germinated root tips were removed and fixed in Carney's solution II (ethanol and acetic acid in 3:1 v/v) for 24 hrs. The Carney's solution II was decanted carefully and the root tips were washed with preservating solvent (70% ethanol). The fixed root tips were persevered in 70% ethanol in refrigerator. The root tips were taken on a clean watch glass and stained with acetoorcein and 1 N HCl solution (7:1 v/v). The watch glasses were warmed up and kept aside for 1 hr. Then the roots were taken on a clean glass slide and squashed by using 45% acetic acid solution followed by the method of Levan [15]. A microscope cover glass was mounted on the material and then the pressure was applied on a cover glass to make sure uniform spreading. Then the cover glass was shielded with molten paraffin wax and the slide was observed under the microscope. The Mitotic Index (M.I) was calculated by the method of Fissceja [16]. The mitotic index was calculated by examining minimum of zone cells. Three replicates were completed for each calculation. The slides were observed under microscope and the photograph has been taken.

$$M.I. = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

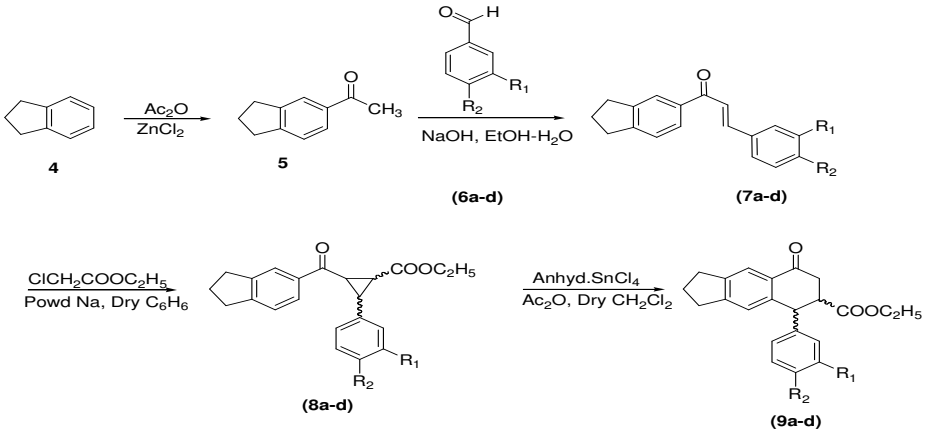
The percent of number of dividing cells compared to the control and the percent inhibition of mitosis by the sample at different concentrations of 100, 200, and 400 ppm against a control were calculated. The concentration required for 50% inhibition (ID_{50}) was extrapolated from the graph of concentration verses percent inhibition according to the method of Hakala [17]. The ID_{50} values of synthesized new tetralone esters for their antimitotic activities were determined and tabulated in table 1.

3. RESULTS AND DISCUSSION

3.1. Chemistry

In this paper, the four steps chalcone route has been followed with some changes in experimental procedure to synthesize new tetralone esters **(9a-d) (Scheme - 1)**. In first step, 5-Acetyl indane **(5)** was prepared in good yields by Friedel-Crafts acylation reaction of Indane **(4)**

with acetic anhydride in the presence of fused zinc chloride catalyst [18, 19]. The structure of acetylindane was confirmed by IR and 1H NMR spectra. The IR spectra showed C=C stretching frequency at 1597 cm^{-1} and C=O stretching frequency at 1672 cm^{-1}. The 1H NMR spectra signals corresponding to COCH3 appeared at 2.58-2.60 ppm. In second step, chalcones **(7a-d)** were prepared in excellent yields by Claisen-Schmidt reaction of 5-Acetyl Indane **(5)** with benzaldehydes **(6a-d)** in the presence of sodium hydroxide base in water-ethanol mixture [20, 21]. The structures of chalcones were confirmed by IR and 1H NMR spectra. The IR spectra of chalcones showed C=C stretching frequency in the range of 1591-1598 cm^{-1} and C=O stretching frequency in the range of 1661-1669 cm^{-1}. The 1H NMR spectra signals corresponding to α-CH and β-CH of chalcones appeared at 7.51-7.57 ppm and 7.74-8.06 ppm with coupling constant J = 12 Hz. In third step, cyclopropyl keto esters **(8a-d)** were prepared in good yields by the reaction of chalcones **(7a-d)** with ethyl chloroacetate in the presence of powdered sodium in dry benzene [22]. The IR spectra showed stretching frequencies at 1595-1598 cm^{-1}, 1671-1679 cm^{-1} and 1741-1745 cm^{-1} for C=C, C=O and ester C=O of cyclopropyl keto esters respectively. The 1H NMR spectra signals of cyclopropyl CH protons appeared at 1.91-2.74 ppm. In fourth step, tetralone esters **(9a-d)** were prepared in very good yields by intramolecular cyclization of cyclopropyl keto esters **(8a-d)** in the presence of anhyd.Stannic chloride and acetic anhydride in dry dichloromethane [23]. Finally the synthesized tetralone esters were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and elemental analysis data. The IR stretching frequencies of



R_1: **a**= H, **b**=H, **c**=H, **d**=CH_3; R_2: **a**=CH_3, **b**=OCH_3, **c**=SCH_3, **d**=CH_3

Scheme - 1: Synthesis of new tetralone esters

Table - 1: Antimitotic activities of the compounds 9a-d by onion root tip method

Compound No.	Conc. in ppm	% Dividing cells	%Dividing cells compared to control	%Inhibition compared to control	ID_{50} in ppm
		24 hrs	24 hrs	24 hrs	24 hrs
Control		31.12	100	0	-
	100	17.03	54.72	45.28	
9a	200	18.62	59.53	40.47	530
	400	16.7	53.66	46.34	
	100	9.42	64.55	35.45	
9b	200	9.23	54.62	45.38	260
	400	9.11	71.78	28.22	
	100	8.95	34.25	65.75	
9c	200	8.54	37.72	62.28	440
	400	8.12	47.55	52.45	
	100	7.25	63.75	36.25	
9d	200	6.82	55.71	44.29	270
	400	5.13	68.76	31.24	

compounds **(9a-d)** showed at 1593-1596 cm^{-1}, 1691-1699 cm^{-1} and 1742-1746 cm^{-1} for C=C, C=O and ester C=O respectively. The 1H NMR spectra signals appeared at 1.17-1.31 ppm and 4.11-4.23 ppm for CH3 and CH2 protons of tetralone esters with a coupling constant J = 4 Hz.

3.2. Antimitotic activity

The *allium cepa* has been used to evaluate the antimitotic activity of novel tetralone esters of podophyllotoxin analogues. The root tip cells in compounds 9a-d exhibited changes in cellular morphology such as slight elongation in shape with many of them remain in the earliest stages of mitosis called prophase stage. Onion roots in compounds 9a-d of 100, 200 and 400 ppm at 24 hrs exhibited changes in chromosomes and shape of the cells with elongated appearance. Using cytotoxic nature of novel tetralone esters showed very less number of dividing cells. Change in chromosomes and cellular morphology were achieved in increasing concentration for 24 hrs. Treatment of root meristem with compounds 9a-d exhibited less change in cell shape with elongated appearance. The result showed that, the percentage of inhibition increases at 24 hrs., compared to control. Meanwhile at 100 ppm, the percentage of inhibition of the germinated root tips in compounds 9a-d is little above the value at 200 and 400 ppm for 24 hrs. Table 1 shows the effect of concentration and time on the cell division of *Allium cepa*. The result shows that the percentage of inhibition of compound 9c was significantly highest with 100 ppm at 24 hrs. Compound 9b showed least inhibition while 9a and 9d exhibited moderate inhibition compared to the control. From the above observations, the partial-c-mitosis, full-c-mitosis with partially functional spindles and comparatively normal mitotic cells phases, chromosomal bridge and chromosomal breakage were noticed in various cells of the same root tip at 24 hrs time. Therefore the antimitotic ability of novel tetralone esters was remarkable in controlling the cell division and acts as very good antimitotic agents. The results of antimitotic activity are given in table 1.

4. CONCLUSION

In conclusion, the new tetralone esters **(9a-d)** were prepared in good yields from chalcone route and they were screened for their antimitotic activities. All the synthesized compounds were identified as good antimitotic agents by onion root tip method. Among these, compound **9c** showed more inhibitions for all the three concentrations, compound **9a** exhibited good inhibitions, compounds **9b** and **9d** exhibited some moderate inhibitions.

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