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Synthesis and evaluation of antimitotic activity of new tetralone acid analogues of podophyllotoxin

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ABSTRACT

The new tetralone acids (7a-e) were synthesized as analogues of podophyllotoxin. They were prepared by replacing 3,4,5-trimethoxyphenyl ring with cyclohexyl group in podophyllotoxin and 1,3-methylene dioxy ring with methoxy, hydrogen, methyl, thiomethyl, and fluorine atoms. The analogues of podophyllotoxin were synthesized using Gensler's method with some changes in reagents and experimental procedure. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis data. The synthesized tetralones acids were screened for their antimitotic activity. It is noteworthy that compound 7b possessed excellent antimitotic activity, 7c and 7e showed considerable activity and remaining 7a and 7d possessed low activity.

1. Introduction

Podophyllotoxin [1] (Figure 1) and its several analogues are being used as cytotoxic spindle poisons and antitumor agents at clinical levels [2]. Several analogues of the podophyllotoxin have been reported in literature. Podophyllotoxin has been extracted from two important medicinal plants named *podophyllum emodi* an Indian species and *podophyllum peltatum*, a North American species [3]. It also occurs in many other plants of podophyllum species. It belongs to the family of natural products called lignans. Podophyllotoxin showed other biological activities such as cathartic, antitropical skin disease, antimalarial, anti-HIV (AIDS) *etc*. [4].

In view of the above facts, it was decided to modify the structure of podophyllotoxin and synthesized tetralone acids as analogues [5-8]. They were synthesized by replacing 3,4,5-trimethoxyphenyl ring with cyclohexyl group in podophyllotoxin and 1,3-methylene dioxy ring with methoxy, methyl, thiomethyl group and hydrogen and fluorine elements and lactone ring with carboxylic acid group. The analogues of podophyllotoxin were synthesized using Gensler's method [9] with some changes in reagents and experimental procedure. The synthesized tetralone acids were screened for their antimitotic activity by onion root method [10].

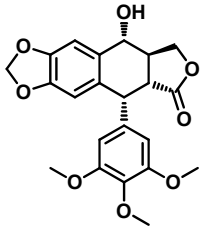

Figure 1. The structure of podophyllotoxin.

2. Experimental

2.1. Materials and methods

All the reagents and chemicals were purchased. They were used without further purification. Melting points were taken in open capillary tubes and are uncorrected. Thin layer chromatography (TLC) is performed with E. Merck pre-coated silica gel plates ($60F_{254}$). Acme, India silica gel, 60-120 mesh is used for column chromatography. IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers.

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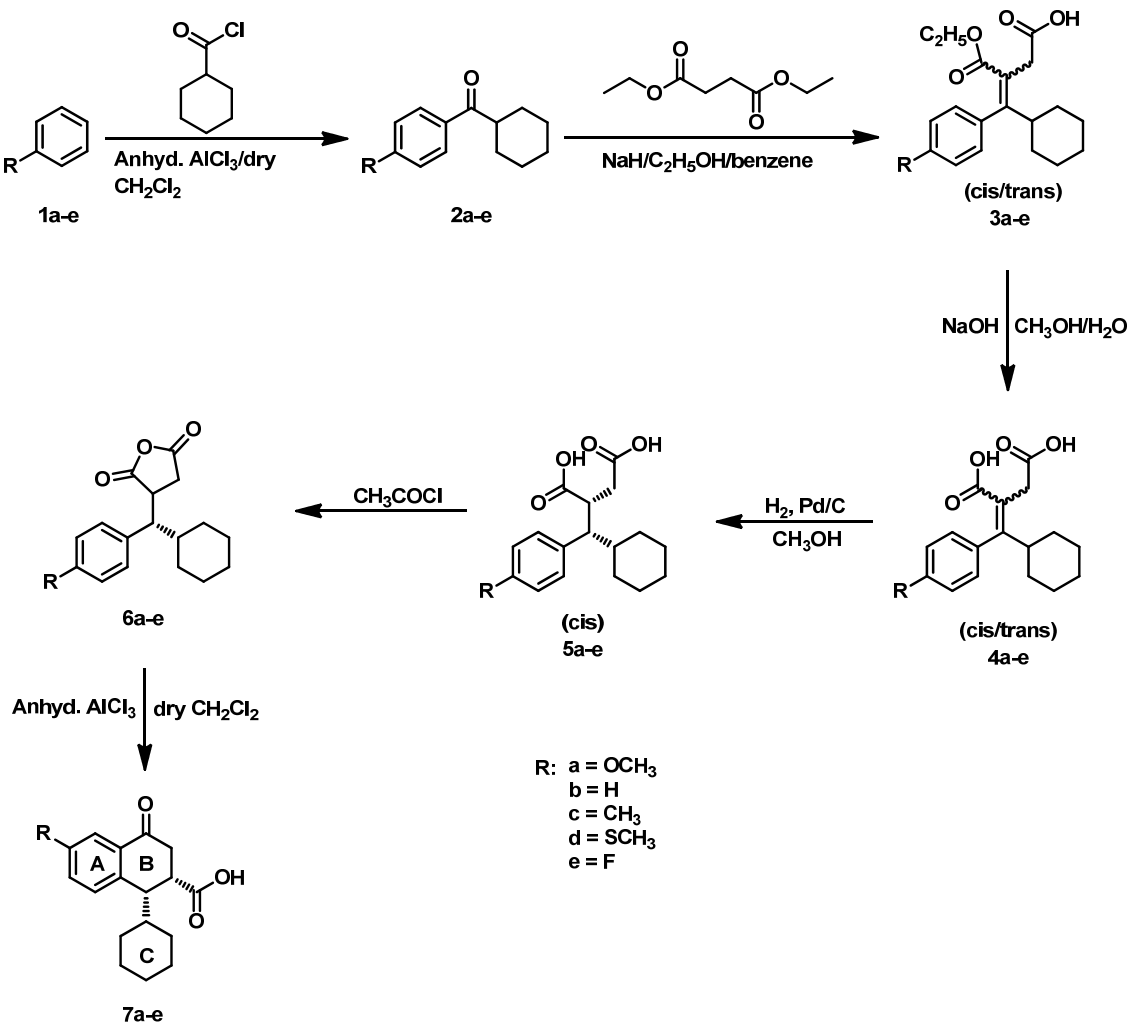

Scheme 1

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using tetramethyl silane (TMS) as an internal reference on Bruker spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400. Mass spectra were obtained by Water-Q-TOF ultima spectrometer. Micro analytical data were obtained by Elemental-Vario EL-III.

2.2. Synthesis

2.2.1. Procedure for the preparation of substituted cyclohexyl(phenyl)methanone (2a-e)

A solution of cyclohexane carbonyl chloride (16.2 g, 0.111 mol) in dichloromethane (50 mL) was added drop wise to the stirred suspension of substituted benzene (**1a-e**) (10 g, 0.092 mol) and anhydrous aluminium chloride (13.48 g, 0.101 mol) in dichloromethane (50 mL) at 0-5 °C under nitrogen gas atmosphere. The reaction mixture was stirred for 5 h at 25-30 °C. The completion of the reaction was known by TLC. The reaction mass was poured into 1 N HCl. The mixture was stirred for 2 h at 25-30 °C. The product was extracted into dichloromethane. The combined organic layer was washed with aq. sodium bicarbonate solution and then with water, dried over anhydrous Na_2SO_4. The organic layer was concentrated under vacuum using rotary evaporator. The products were recrystallized from diethyl ether (Scheme 1).

Cyclohexyl(4-methoxyphenyl)methanone (**2a**): Color: White solid. Yield: 74.40 %. M.p.: 61-63 °C. IR (KBr, ν, cm^{-1}): 2926 (C-H), 2973 (Ar-H), 1738 (ester, C=O), 1697 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.20-1.80 (m, 10H, cyclic -CH_2), 2.23-2.65 (p, 1H, cyclic-CH), 3.78 (s, 3H, OCH_3), 6.90-7.90 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.1, 25.6, 25.9, 29.1, 29.6, 47.3, 55.7, 114.1, 114.5, 129, 129.2, 129.4, 165.4, 202.3. MS (ESI, *m/z*): 219.10 (M^+). Anal. calcd. for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 77.01; H, 8.27%.

Cyclohexyl(phenyl)methanone (**2b**): Color: White crystalline solid. Yield: 71.91 %. M.p.: 52-54 °C. IR (KBr, ν, cm^{-1}): 1685 (C=O), 2995 (Ar CH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.10-1.23 (m, 10H, cyclic -CH_2), 2.14-2.68 (p, 1H, cyclic -CH), 6.70-7.40 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.0, 25.3, 25.7, 29.2, 29.7, 47.9, 128, 128.2, 128.4, 128.9, 133.4, 139.4, 220.5. MS (ESI, *m/z*): 189.06 (M^+). Anal. calcd. for $C_{13}H_{16}O$: C, 82.94; H, 8.57. Found: C, 82.91; H, 8.54%.

Cyclohexyl(p-tolyl)methanone (**2c**): Color: White solid. Yield: 70.15 %. M.p.: 71-73 °C. IR (KBr, ν, cm^{-1}): 1696 (C=O), 2927 (Methyl CH), 3030 (Ar CH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.93-1.93 (m, 10H, cyclic -CH_2), 2.38 (s, 3H, -CH_3), 2.64 (p, 1H, cyclic -CH), 6.82-7.37 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.9, 25.1, 25.6, 25.7, 29.1, 29.3, 47.8, 128.1,

128.4, 128.7, 128.9, 133.5, 142.6, 202.5. MS (ESI, *m/z*): 203.10 (M^+). Anal. calcd. for $C_{14}H_{18}O$: C, 83.12; H, 8.97. Found: C, 83.09; H, 8.95%.

Cyclohexyl(4-(methylthio)phenyl)methanone (**2d**): Color: White solid. Yield: 75.05 %. M.p.: 87-89 °C. IR (KBr, ν, cm^{-1}): 1706 (C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.44-1.80 (m, 10H, cyclic -CH_2), 2.56 (p, 1H, cyclic -CH), 2.54 (s, 3H, -SCH_3), 6.70-7.26 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.7, 25.2, 25.5, 25.8, 29.4, 29.7, 47.9, 126.4, 126.9, 132.1, 132.6, 133.4, 143.5, 202.4. MS (ESI, *m/z*): 235.02 (M^+). Anal. calcd. for $C_{14}H_{18}OS$: C, 71.75; H, 7.74. Found: C, 71.73; H, 7.70%.

Cyclohexyl(4-fluorophenyl)methanone (**2e**): Color: White gummy mass. Yield: 70.21 %. IR (KBr, ν, cm^{-1}): 1690 (C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.13-1.87 (m, 10H, cyclic -CH_2), 2.76 (p, 1H, cyclic -CH), 7.13-7.74 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25, 25.3, 25.9, 29.2, 29.5, 47.9, 115.4, 115.7, 130.2, 130.7, 132.9, 167.7, 202.8. MS (ESI, *m/z*): 206.08 (M^+). Anal. calcd. for $C_{13}H_{15}FO$: C, 75.70; H, 7.33. Found: C, 75.68; H, 7.30%.

2.2.2. General procedure for the synthesis of substituted 2-(cyclohexyl(phenyl)methylene)succinic acid (4a-e)

Sodium hydride (2.47 g, 0.103 mol) was added in portions to the stirred suspension of substituted cyclohexyl(phenyl) methanone (**2a-e**) (5 g, 0.0229 mol) and diethyl succinate (11.9 g, 0.068 mol) in dry benzene (15 mL) under nitrogen gas atmosphere. The reaction mixture was stirred for 10 min at 25-30 °C (until the evolution of hydrogen gas ceased). Absolute ethanol (5.2 g, 0.114 mol) was added drop wise during 1 h to the above solution. The reaction mass was stirred at 25-30 °C for 15 h. The completion of the reaction was confirmed by TLC. The reaction mixture was cooled and acidified by addition of glacial acetic acid. The substituted succinic half esters were extracted into ether and then into saturated sodium carbonate solution. The sodium carbonate extract was neutralized by dilute acetic acid. The precipitated gummy residue was extracted into dichloromethane. The organic layer was washed with water and concentrated under vacuum using rotary evaporator to give white solid in good yields. The substituted succinic acid half esters (**3a-e**) (4 g, 0.0115 mol) were added to the stirred solution of methanol (15 mL), and sodium hydroxide solution (15 mL) (2.3 g, 0.057 mol). The resultant reaction mixture was refluxed at 75-80 °C for 5 h. Here again the completion of the reaction was assessed with TLC. The reaction mixture was concentrated to give slight yellow residue. The resultant residue was dissolved with 40 mL water. The aqueous layer was neutralized with dilute hydrochloric acid to obtain gummy light brownish mass and extracted to dichloromethane. The separated organic layer washed with water, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was dissolved in 30 mL diethyl ether and kept overnight in fridge. The products precipitated as white needle type crystals and were filtered to afford pure compounds in good yields (Scheme 1).

2-(Cyclohexyl(4-methoxyphenyl)methylene)succinic acid (**4a**): Color: white crystal solid. Yield: 88.10 %. M.p.: 165-167 °C. IR (KBr, ν, cm^{-1}): 3500 (carboxylic OH), 1713.8 (CH_2-C=O), 1663 (α, β unsaturated C=O), 1605 (Ar C=C). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.14-1.62 (m, 10H, cyclic -CH_2), 2.40 (p, 1 H, cyclic -CH), 3.62 (s, 2H, -CH_2), 3.78 (s, 3H, -OCH_3), 6.90-7.33 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.1, 25.7, 26, 26.2, 26.5, 32.5, 34.8, 55.5, 114, 117.5, 129.5, 129.8, 131.4, 155.1, 159.8, 171.6, 174.4. MS (ESI, *m/z*): 318.11 (M^+). Anal. calcd. for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97. Found: C, 67.89; H, 6.94%.

2-(Cyclohexyl(phenyl)methylene)succinic acid (**4b**): Color: Light yellow solid. Yield: 78.05 %. M.p.: 159-161 °C. IR (KBr, ν, cm^{-1}): 3400-3200 (carboxylic OH), 1603 (Ar C=C), 1701 (CH_2-C=O), 1660 (α, β unsaturated C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.89-1.63 (m, 10H, cyclic -CH_2), 2.36 (p, 1H, cyclic -CH), 3.70 (s, 2H, -CH_2), 6.84-7.3 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.4, 25.7, 26, 26.1, 26.4, 32.9, 34.4, 117.7, 126.2, 126.9, 127.8, 128.4, 128.8, 139.4, 155.8, 171.9, 174.4. MS (ESI, *m/z*): 289.13 (M^+). Anal. calcd. for $C_{17}H_{20}O_4$: C, 70.81; H, 6.99. Found: C, 70.78; H, 6.95%.

2-(Cyclohexyl(p-tolyl)methylene)succinic acid (**4c**): Color: Light yellow solid. Yield: 80.15 %. M.p.: 172-174 °C. IR (KBr, ν, cm^{-1}): 3500 (cyclic OH), 1600 (Ar C=C), 1680 (CH_2-C=O), 1664 (α, β unsaturated C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.96-1.55 (m, 10H, cyclic -CH_2), 2.36-2.54 (p, 1H, cyclic -CH), 2.56 (s, 3H, -CH_3), 3.62 (s, 2H, -CH_2), 7.10-7.59 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.7, 25.1, 25.8, 26.1, 26.4, 26.7, 32.1, 34.7, 117.7, 126.2, 126.7, 128.4, 128.9, 136.1, 137.4, 155.1, 171.4, 174.2. MS (ESI, *m/z*): 303.15 (M^+). Anal. calcd. for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.49; H, 7.31%.

2-(Cyclohexyl(4-(methylthio)phenyl)methylene)succinic acid (**4d**): Color: Light yellow solid. Yield: 92.61 %. M.p.: 147-149 °C. IR (KBr, ν, cm^{-1}): 3435 (carboxylic OH), 1685 (CH_2-C=O), 1663 (α, β unsaturated C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.13-1.65 (m, 10H, cyclic -CH_2), 2.10 (p, 1H, cyclic -CH), 2.53 (s, 3H, SCH_3), 3.63 (s, 2H, CH_2) 7.30-7.20 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.5, 25.1, 25.7, 26.2, 26.4, 26.8, 32.1, 34.4, 117.8, 126.4, 126.7, 127.4, 126.9, 135.8, 138.3, 155.5, 171.5, 174.6. MS (ESI, *m/z*): 335.04 (M^+). Anal. calcd. for $C_{18}H_{22}O_4S$: C, 64.65; H, 6.63. Found: C, 64.63; H, 6.61%.

2-(Cyclohexyl(4-fluorophenyl)methylene)succinic acid (**4e**): Color: Light yellow gummy mass. Yield: 92.94 %. IR (KBr, ν, cm^{-1}): 3450 (carboxylic OH), 1685 (CH_2-C=O), 1662 (α, β unsaturated C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.93-1.65 (m, 10H, cyclic -CH_2), 2.27 (p, 1H, cyclic -CH), 3.62 (s, 2H, -CH_2), 6.84-7.30 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.1, 25.3, 25.3, 26, 26.3, 26.4, 32.5, 34.4, 115.1, 115.7, 117, 128.4, 128.9, 135.4, 155.1, 135.4, 155.1, 162.4, 171.4, 174.9. MS (ESI, *m/z*): 306.03 (M^+). Anal. calcd. for $C_{17}H_{19}FO_4$: C, 66.65; H, 6.25. Found: C, 66.63; H, 6.21%.

2.2.3. General procedure for the preparation of substituted 2-(cyclohexyl(phenyl)methyl)succinic acid (5a-e)

In an autoclave vessel, substituted 2-cyclohexyl(phenyl methylenesuccinic acid (**4a-e**) (3 g, 0.0094 mol) in methanol (30 mL) and 10% Pd/C (0.3 g) were taken under nitrogen gas atmosphere. The reaction mixture was hydrogenated at 3-4 kg/cm^3 hydrogen pressure in the presence of palladium over carbon at 25-30 °C for 3-4 h. The Pd/C was filtered through hyflobed under nitrogen gas atmosphere. The mixture was concentrated under reduced pressure. The products were recrystallized from ethanol (Scheme 1).

2-(Cyclohexyl(4-methoxyphenyl)methyl)succinic acid (**5a**): Color: White solid. Yield: 78.21 %. M.p.: 152-154 °C. IR (KBr, ν, cm^{-1}): 3460 (carboxylic OH), 1708 (CH_2-C=O), 1660 (α, β unsaturated C=O), 1590 (Ar C=C). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.86-1.52 (m, 10H, cyclic -CH_2), 1.80-2.24 (m, 1H, cyclic -CH), 2.63 (d, 2H, -CH_2), 3.06-3.38 (m, 2H, CH-CH), 3.76 (s, 3H, OCH_3), 6.80-7.23 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 26.2, 26.3, 26.6, 31.2, 31.4, 36.5, 41.7, 55.7, 114.2, 114.4, 129.4, 129.7, 133.7, 157.8, 173.4, 178.6. MS (ESI, *m/z*): 321.16 (M^+). Anal. calcd. for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found: C, 67.47; H, 7.52%.

2-(Cyclohexyl(phenyl)methyl)succinic acid (**5b**): Color: White gummy mass. Yield: 78.4 %. IR (KBr, ν, cm^{-1}): 3502 (carboxylic OH), 1700 (CH_2-C=O), 1658 (α, β unsaturated C=O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.93-1.74 (m, 10H, cyclic -CH_2), 1.90-2.32 (m, 1H, cyclic -CH), 3.64 (d, 2H, -CH_2), 3.10-3.30 (m, 2H, CH-CH), 6.90-7.30 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 26.3, 26.4, 26.8, 30.2, 31.1, 31.9, 36.9, 37.4, 41.4, 125.9, 128.1, 128.2, 128.3, 128.4, 141.3, 173.8, 178.4, 173.8, 178.4. MS (ESI, *m/z*): 291.10 (M^+). Anal. calcd. for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.31; H, 7.61%.

2-(Cyclohexyl(p-tolyl)methyl)succinic acid (**5c**): Color: White solid. Yield: 96.47 %. M.p.: 180-183 °C. IR (KBr, ν, cm^{-1}): 3440 (carboxylic OH), 1709 (CH_2-C=O), 1666 (α, β unsaturated C=O),

2920 (methyl CH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.10-1.40 (m, 10H, cyclic -CH_2), 1.83-2.27 (m, 1H, cyclic -CH), 2.54 (s, 3H, -CH_3), 3.68 (d, 2H, -CH_2), 3.03-3.32 (m, 2H, CH-CH), 6.93-7.44 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 178.3, 173.4, 138.6, 135.4, 128.9, 128.7, 128.4, 128.1, 41.7, 37.4, 36.5, 31.4, 31.2, 30.0, 26.4, 26.3, 26.1, 21.6. MS (ESI, *m/z*): 305.09 (M^+). Anal. calcd. for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 71.00; H, 7.92 %.

2-(Cyclohexyl(4-(methylthio)phenyl)methyl)succinic acid (**5d**): Color: White gummy mass. Yield: 73.89 %. IR (KBr, ν, cm^{-1}): 3405 (carboxylic OH), 1692 (CH_2-C=O), 1663 (α, β unsaturated C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.83-1.45 (m, 10H, cyclic CH_2), 1.80-2.36 (m, 1H, cyclic -CH), 2.56 (s, 3H, SCH_3), 3.62 (d, 2H, -CH_2), 3.10-3.46 (m, 2H, CH-CH), 7.16-7.47 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.1, 26.6, 26.4, 30, 31.2, 31.4, 36.5, 37.4, 41.7, 128.1, 128.4, 128.7, 128.9, 135.4, 138.6, 173.4, 178.9. MS (ESI, *m/z*): 337.01 (M^+). Anal. calcd. for $C_{18}H_{24}O_4S$: C, 64.26; H, 7.19. Found: C, 64.24; H, 7.15%.

2-(Cyclohexyl(4-fluorophenyl)methyl)succinic acid (**5e**): Color: White gummy mass. Yield: 75.35 %. IR (KBr, ν, cm^{-1}): 3495 (carboxylic OH), 1708 (CH_2-C=O), 1664 (α, β unsaturated C=O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.83-1.49 (m, 10H, cyclic -CH_2), 1.86-2.23 (m, 1H, cyclic -CH), 3.53 (d, 2H, -CH_2), 3.11-3.34 (m, 2H, CH-CH), 6.90-7.36 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.2, 26.1, 26.4, 26.7, 30.4, 31.3, 31.6, 36.4, 37.4, 41.5, 128, 128.2, 128.4, 128.8, 138.4, 139.4, 178.9. MS (ESI, *m/z*): 309.11 (M^+). Anal. calcd. for $C_{17}H_{21}FO_4$: C, 66.22; H, 6.86. Found: C, 66.19; H, 6.82%.

2.2.4. General procedure for the preparation of 1-cyclohexyl-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (7a-e)

Substituted 2-(cyclohexyl(phenyl)methyl)succinic acid (**5a-e**) (3 g, 0.00937 mol) and acetyl chloride (30 mL) mixture was refluxed for 6 h. The completion of the reaction was known by TLC. The excess of the acetyl chloride was distilled off under atmospheric pressure. The resultant mixture was dissolved in dichloromethane (25 mL) and washed with 5% cold sodium bicarbonate solution. The separated organic layer was finally washed with water and dried over anhyd sodium sulphate. Distilled off solvent to afford a gummy mass (**6a-e**) in good yield (Scheme 1).

Above compounds (**6a-e**) (1 g, 0.0033 mol) were dissolved in dichloromethane (10 mL) and added dropwise to the stirred suspensions of anhydride aluminum chloride (1.1 g, 0.0082 mol) in dichloromethane (10 mL) at 0-5 °C under nitrogen gas atmosphere. The reaction mixture was stirred for 6h at 25-30 °C. The completion of the reaction was known by TLC. The reaction mass was poured into 1 N HCl (30 mL). The product was extracted into dichloromethane and then into saturated Na_2CO_3 solution. The sodium carbonate extracts were acidified by dil. HCl to give products in good yields. They were recrystallized from ethanol (Scheme 1).

1-Cyclohexyl-6-methoxy-4-oxo-1,2,3,4-tetrahydro naphthalene-2-carboxylic acid (**7a**): Color: Yellow solid. Yield: 66.18 %. M.p.: 199-201 °C. IR (KBr, ν, cm^{-1}): 3396 (carboxylic OH), 1715 (carboxylic C=O), 1702 (tetralone C=O), 1597 (Ar C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.89-1.30 (m, 10H, cyclic -CH_2), 1.43-1.76 (m, 1H, cyclic -CH), 2.37 (dd, 2H, -CH_2), 3.02 (t, 1H, CH), 3.46 (q, 1H, -CH), 3.78 (s, 3H, -OCH_3), 7.10-7.44 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 26.0, 26.1, 26.4, 31.2, 36.4, 37.5, 39.4, 55.3, 108.8, 122.8, 129.3, 130.4, 134.7, 157.6, 178.5, 196.4. MS (ESI, *m/z*): 303.12 (M^+). Anal. calcd. for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.47; H, 7.31%.

1-Cyclohexyl-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (**7b**): Color: Yellow solid. Yield: 64.90 %. M.p.: 170-172 °C. IR (KBr, ν, cm^{-1}): 3435 (carboxylic OH), 1701 (tetralone C=O), 1716 (carboxylic C=O), 1595 (Ar C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.93-1.68 (m, 10H, cyclic -CH_2), 1.75-1.97 (m, 1H, cyclic -CH), 2.37 (dd, 2H, -CH_2), 2.87 (t, 1H, -CH), 3.23-3.47 (q, 1H, CH), 6.87-7.43 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 26.2, 26.3, 26.5, 31.5, 31.7, 31.3, 36.5, 37.5, 38.4, 39.7, 123.8, 125.6, 127.2, 131.6, 132.4, 138.5, 178.4, 196.5. MS (ESI, *m/z*): 272.05 (M^+). Anal. calcd. for $C_{17}H_{20}O_3$: C, 74.97; H, 7.40. Found: C, 74.93; H, 7.37%.

1-Cyclohexyl-6-methyl-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (**7c**): Color: Yellow solid. Yield: 61.81 %. M.p.: 184-186 °C. IR (KBr, ν, cm^{-1}): 3435 (carboxylic OH), 1705 (tetralone C=O), 1718 (carboxyl C=O), 1714 (carboxylic C=O), 1594 (Ar C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.13-1.53 (m, 10H, cyclic -CH_2), 1.80-2.17 (m, 1H, cyclic -CH), 2.32 (s, 3H, -CH_3), 2.50 (dd, 2H, CH_2), 2.93-3.24 (t, 1H, CH), 3.67-3.83 (q, 1H, CH), 6.86-7.42 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.8, 26.2, 26.3, 26.4, 31.2, 31.6, 36.5, 37.6, 38.7, 39.5, 124.7, 127.6, 133.5, 135.4, 135.6, 140.5, 178.6, 196.8. MS (ESI, *m/z*): 287.06 (M^+). Anal. calcd. for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.48; H, 7.71%.

1-Cyclohexyl-6-(methylthio)-4-oxo-1,2,3,4-tetrahydro naphthalene-2-carboxylic acid (**7d**): Color: Yellow solid. Yield: 67.72 %. M.p.: 161-163 °C. IR (KBr, ν, cm^{-1}): 3440 (carboxylic OH), 1710 (tetralone C=O), 1718 (carboxyl C=O), 1587 (Ar C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.13-1.44 (m, 10H, cyclic -CH_2), 1.86-2.18 (m, 1H, cyclic -CH), 2.49 (s, 3H, SCH_3), 2.61 (dd, 2H, -CH_2), 3.07 (t, 1H, CH), 3.53-3.72 (q, 1H, -CH_2), 7.06-7.48 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.3, 26.3, 26.4, 26.8, 31.3, 31.5, 36.4, 37.6, 38.4, 39.5, 124.3, 128.9, 132.8, 134.7, 135.9, 136.7, 178.7, 196.5. MS (ESI, *m/z*): 319.03 (M^+). Anal. calcd. for $C_{18}H_{22}O_3S$: C, 67.89; H, 6.96. Found: C, 67.87; H, 6.92%.

1-Cyclohexyl-6-fluoro-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (**5e**): Color: Yellow solid. Yield: 66.94 %. M.p.: 201-203 °C. IR (KBr, ν, cm^{-1}): 3390 (carboxylic OH), 1703 (tetralone C=O), 1720 (carboxyl C=O), 1599 (Ar C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.96-1.46 (m, 10H, cyclic -CH_2), 1.63-1.85 (m, 1H, cyclic -CH), 2.42 (dd, 2H, CH_2), 2.83-3.15 (t, 1H, CH), 3.42-3.68 (q, 1H, CH), 7.18-7.69 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 26.0, 26.3, 26.7, 31.4, 31.8, 36.1, 37.5, 38.6, 39.4, 113.4, 119.9, 129.4, 134.6, 135.4, 160.6, 178.8, 196.5. MS (ESI, *m/z*): 291.01 (M^+). Anal. calcd. for $C_{17}H_{19}FO_3$: C, 70.33; H, 6.60. Found: C, 70.30; H, 6.57%.

2.3. Antimitotic study

The novel tetralone acid analogues of podophyllotoxin (**7a-e**) were screened for antimitotic activity by onion root tip method and the ID_{50} was determined. The materials required are acetoorcein solution, compound microscope, glass slides, cover slips, hydrochloric acid (0.1 N), Carney's solution II, 70% ethanol and tested samples (100, 200 and 400×10^{-6} mol/L). To study the effect of novel tetralone acids analogues of podophyllotoxin on somatic cells, onion base was immersed to an extent of about half a centimeter in a sample tube and control solution tube (7×3), after removing the old roots from them and immersion is continued for 24 h. for germination. After different time intervals, the germinated root tips were removed and were fixed in Carney's solution II (alcohol and acetic acid in 3:1 ratio respectively) for 24 h. After 24 h. Carney's solution II was decanted carefully and the root tips were washed with preserving solvent (70% ethanol). The fixed root tips were preserved in 70% ethanol in refrigerator. The root tips were taken in watch glass and stained with a drop of acetoorcein stain and a drop of 1 N HCl (7:1, *v:v*). The glasses were warmed and kept for 1 hr. The roots were taken on a clean glass slide and squashed using 45% acetic acid following the method of Levan [11]. A microscope cover glass was placed on the material and then pressure was applied on a cover glass to ensure uniform spreading. The cover glass was sealed with molten paraffin wax and slide was observed under microscope.

Table 1. Antimitotic activity of the compounds (**7a-e**) by onion root tip method.

Compound	Concentration ($\times 10^{-6}$ mol/L)	% Dividing Cell	% Dividing cells compared to control	% Dividing inhibition compared to control	IC_{50} ($\times 10^{-6}$ mol/L)
7a	2.03	7.513	35.57	64.43	1.66
	3.02	5.981	28.32	71.68	
	4.01	4.736	22.42	77.58	
7b	2.03	13.01	61.60	38.40	9.12
	3.04	13.45	63.68	36.32	
	4.02	13.87	65.67	34.33	
7c	2.01	11.06	52.37	47.63	6.42
	3.03	10.68	50.57	49.43	
	4.04	10.21	48.34	51.66	
7d	2.03	06.34	30.02	69.98	1.58
	3.04	05.12	24.24	75.76	
	4.02	04.06	19.22	80.78	
7e	2.01	08.06	38.16	61.84	5.83
	3.02	08.67	41.05	58.95	
	4.05	08.01	37.93	62.07	
Control	-	21.12	100	00.00	0.00

Mitotic Index (MI) was calculated by following method of Fissceja [12]. The mitotic index was determined by examination of minimum of zone cells. Three replicates were made for each calculation. The slides were observed under microscope and photographed.

$$M.I. = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100 \quad (1)$$

The percentage of the number of dividing cells compared to the control and the percent inhibition of mitosis by antimitotic agent at a different concentration such as 100, 200, and 400×10^{-6} mol/L against a control were calculated. The concentration needed for 50% inhibition (ID_{50}) was extrapolated from the graph of the concentration verses percentage inhibition. ID_{50} values for novel tetralone acid analogues of podophyllotoxin for antimitotic activity were calculated individually following hakala [13] method.

3. Results and discussion

3.1. Chemistry

The tetralone acid analogues of podophyllotoxin were synthesized by gensler's method (Scheme 1). Substituted cyclohexyl(phenyl)methanone (**2a-e**) were prepared in high yields by stirring an equimolar solution of substituted benzenes separately with cyclohexyl carbonyl chloride in the presence of anhyd. aluminium chloride in dichloromethane [14]. Itaconic acid half esters (**3a-e**) were prepared as a mixture of cis and trans isomers by Stobbe condensation of substituted cyclohexyl (phenyl)methanone (**2a-e**) with diethyl succinate using sodium hydride as a base in benzene and absolute ethanol at room temperature [15]. Substituted 2-(cyclohexyl(phenyl)methyl-ene)succinic acid (**4a-e**) were prepared by the saponification of itaconic acid half esters by refluxing in methanol and water mixture in the presence of sodium hydroxide. Substituted 2-(cyclohexyl(phenyl)methyl)succinic acid (**5a-e**) were prepared by the catalytic hydrogenation in the presence of palladium over carbon in methanol under 3-4 kg/cm^3 hydrogen pressure at 25-30 °C [16,17]. Benzhydryl succinic anhydrides (**6a-e**) were prepared by refluxing benzhydryl succinic acids with acetyl chloride. Tetralone acids (**7a-e**) were prepared by the intramolcular Friedel-Craft's acylation reaction of benzhydryl succinic anhydrides in the presence of Lewis acids anhyd. aluminium chloride in dichloromethane (Scheme 1) [18,19]. The products were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis data.

Tetralone acids **7a** and **7d** were obtained in good yields (65-68%). The products **7b**, **7c** and **7e** were formed in moderate yields (50-53%). The formation of moderate yield might be due to the less electron donating nature of hydrogen, methyl and fluorine linked phenyl ring. Hence the π-electrons of benzene ring are not readily available for Friedel-Craft's intramaolecular acylation reactions. The NMR spectrum of tetralone acid **7a** showed a triplet at 3.3-3.6 ppm (J = 6 Hz) for the C_1-H. The large coupling constant (J) value indicated that C_1-H and C_2-H in **7a** were diaxial. Hence the C_2-H carbonyl and C_1-H cyclohexyl groups should be trans to each other, a configuration being thermodynamically more stable.

3.2. Antimitotic activity

As regards the relationships between the structure of the podophyllotoxin scaffold and antimitotic properties, it showed varied antimitotic activity (Table 1). The presence of different substituents on the ring A causes a certain changes in activity. The compound **7b** has *hydrogen,* moiety on ring A, which is accounted for the enhanced antimitotic activity than when compared to control solution. Similarly compounds **7c** and **7e** have showed significant activity. The compounds **7c** and **7e** have *methyl* and *fluorine* moiety on ring A, which is accounted for the moderate activity of the compounds. On the other hand, the remaining compounds **7a** and **7d** have showed less activity compared to control. From the obtained results, it is clear that the major role for antimitotic activity is played by substituents on ring A moiety. It is evident that novel tetralone acid analogues of podophyllotoxin were showed good antimitotic activity.

4. Conclusion

In conclusion, the new tetralone acid analogues of podophyllotoxin (**7a-e**) were synthesized in good yields following Gensler's method with some changes in reagents and reaction conditions. The structures of synthesized compounds were confirmed and characterized by analytical data's such as IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and elemental analysis. They were screened for antimitotic activity. The novel tetralone acids analogues of podophyllotoxin were synthesized by replacing methylene dioxy group in podophyllotoxin with methoxy, methyl, thiomethyl group and hydrogen and fluorine and the trimethoxyphenyl ring with cyclohexyl and lactone ring with carboxylic acid to study the structure activity relationship. It is noteworthy that compound **7b** possessed good antimitotic activity, **7c** and **7e** showed considerable activity and remaining **7a** and **7d** possessed low activity.

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