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SYNTHESIS AND EVALUATION OF ANTIMITOTIC ACTIVITY OF NEW TETRALONE ACIDS

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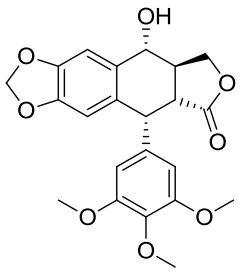
ABSTRACT
The new tetralone acid intermediates were synthesized in high yields for analogues of podophyllotoxin by Gensler's method with slight change in reagents and experimental procedure. All the synthesised compounds were characterized by spectral and elemental analysis data. The synthesized new compounds were evaluated for antimitotic activity by onion root tip method. The synthesized analogues showed strong to moderate activity. The antimitotic activity was compared with control, the compounds **13e** and **13f** were showed good inhibition of normal cell devision and compounds **13a** and **13c** exhibited less inhibition while the compounds **13b** and **13d** exhibited moderate activity.

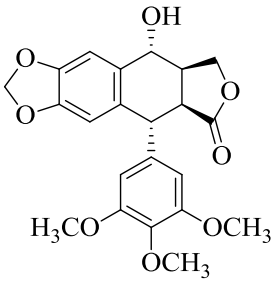
KEYWORDS: Ketones, Itaconic acids, Tetralone acds, Gensler's method, Stobbe condensation, Catalytic hydrogenation, Antimitotic activity.

1. INTRODUCTION
Podophyllotoxin (**1**)[1] is a bioactive lignan. It was isolated from the medicinal plants named Podophyllum emodi and Podophyllum Peltatum and many other plants of Podophyllum species. Podophyllotoxin showed wide variety of biological activities such as cathartic, cytotoxic, antimitotic, anticancer, anti AIDS, antitropical skin disease, antimalarial, virucidal, fungicidal etc.

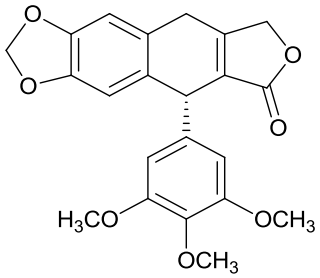
A lot of attention was given to the podophyllotoxin for the synthesis of its derivatives and analogues. Extensive structural modifications, particularly at the C_4 position of podophyllotoxin have been reported in the literature.[2–4], it is readily epimerization to Picropodophyllin (**2**) which is not so active. β-apopicropodophyllin (**3**), a dehydrated isomerised product of Podophyllotoxin exhibit much stronger antimitotic activity.



Podophyllotoxin(**1**)

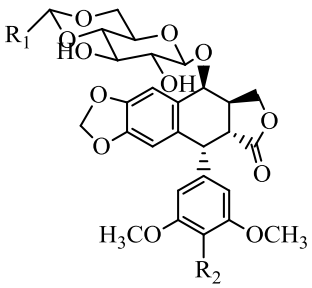


Picropodophyllin(**2**)



β-apopicropodophyllin(**3**)

Etoposide (**4**) and teniposide (**5**) are semisynthetic bioactive derivatives of the natural podophyllotoxin currently used in cancer chemotherapy against various cancers, including small cell lung cancer, testicular carcinoma, lymphoma and Kaposi's sarcoma.[5,6] Some of its derivatives also exhibit cytotoxic, cathartic and anticancer activities.[7-9]



	R_1	R_2
Etoposide(**4**)	$-CH_3$	-OH
Tenoposide(**5**)		-OH

Although podophyllotoxin is an antimicrotubule agent, etopodside and tenoposide inhibit the catalytic activity of DNA topoisomerase II.[10, 11]

In view of the above facts, it was decided to modify the structure of podophyllotoxin and synthesized new tetralone acids.[12-16] They were (**13a-f**) synthesized by replacing methylenedioxy ring with methylthio groups and changing substituents in 3,4,5-trimethoxy Phenyl ring C respectively with benzyl, p-tolyl, p- flourophenyl, p-nitrophenyl, p-methoxyphenyl and cyclohexyl group in **1** and **2** to study the structure activity relationship. The analogues of podophyllotoxin were synthesized using Gensler's method[17] with some changes in reagents and experimental procedure. The synthesized new tetralone acids were screened for their antimitotic activity by onion root method.[18]

2. EXPERIMENTAL

2.1. Materials and methods

All the reagents and solvents were purchased from Merck. 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 precoated silica gel plates (60F-254) with iodine as developing agent.IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers. ^{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.

2.2. Synthesis

2.2.1. General procedure for the synthesis of substituted benzophenones (8a-f)

The thioanisole **6 (**5g, 0.04025mole) and anhydrous stannic chloride (4.7108g, 0.04025mole) were taken in a dry dichloromethane (30ml).The reaction mixture was stirred continuously for about half an hour. A solution of benzoyl chlorides **7a-f** (4.6721g, 0.04025mole) in dry dichloromethane (30ml) was added dropwise over a period of one hour to the above cooled mixture. After 10 hour, the temperature of the reaction mixture has been allowed to become 25^{o}C, Conc. HCl was added dropwise over a period of 30 minutes. The reaction mixture was further stirred for 8 hour. During the addition of HCl, large amount of HCl gas is evolved.

The product was extracted into dichloromethane, washed with 10% aq. NaOH solution (2x50ml) and then with 2% aq. NaCl solution (2x30ml). The solvent was removed by distillation. The product was purified by repeated recrystallization with methanol to afford substituted benzophenones in good yields.

(3-(Methylthio) phenyl) (phenyl) methanone (8a)

Colour: white solid. Yield: 80.52%. m.p. 119-123^{o}C. IR (KBr, ν, cm^{-1}): 1678 (C=O), 1593(aromatic C=C), 3025, 2922 and 2854cm^{-1} was due to C-H stretch of aromatic and SCH_3 group. ^{1}H NMR ($CDCl_3$-400MHz) δ ppm: 2.52 (S, 3H, SCH_3), 7.30-7.79 (m, 9H, Ar-H).

^{13}C NMR (CDC_{l3} -100 MHz) δ ppm: 14.7, 126.4, 128.3, 130.5, 123.1, 134.2, 138.3, 143, 194.5. MS (ESI, m/z): 228.05 (M^+). Anal. Calcd. For $C_{14}H_{12}O_5$; C, 73.65; H, 5.30; O, 7.01; S, 14.04; Found: C, 73.62; H, 5.32%.

(3- (Methyl thio)phenyl)(p-tolyl) methanone (8b)

Colour: orange solid. Yield: 78%. m.p. 119-123^{o}C. IR (KBr, ν, cm^{-1}): 1675 (C=O), 1597(aromatic C=C), 3025, 2922 and 2854cm^{-1} was due to C-H stretch of aromatic and SCH_3, -CH_3groups. ^{1}H NMR (CDC_{l3}-400MHz) δ ppm: 2.32 (s, 3H, CH_3), 2.52(s, 3H, SCH_3), 7.28-7.65(m, 8H, Ar-H). ^{13}C NMR (CDC_{l3} -100 MHz) δ ppm: 14.7, 21.2, 126.5, 128.8, 130.7, 134.2, 135.4, 143, 194.5. MS (ESI, m/z): 242 (M^+). Anal. Calcd. for $C_{15}H_{14}O_5$: C, 74.34; H, 5.82; O, 6.60; S, 13.23. Found: C, 74.31; H, 5.83.

(3- (Methyl thio)phenyl)(4-nitrophenyl) methanone (8c)

Colour: pale yellow crystalline solid. Yield: 76.10%. M.P.: 98-103^{o}C. IR (KBr, ν, cm^{-1}): 1677 (C=O), 1605(aromatic C=C), 2923 and 2853cm^{-1} was due to C-H stretch of aromatic and SCH_3 group. ^{1}H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 7.5-8.3(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 123.5, 126.5, 129.8, 130.6, 134.2, 143, 144, 151.4, 194.5. MS (ESI, m/z): 273 (M^+). Anal. Calcd. for $C_{14}H_{11}NO_3S$; C, 61.52; H, 4.06; N, 5.12; O, 17.56; S, 11.73. Found: C, 61.54; H, 4.03%.

(4-Flurophenyl)(3-methylthio)phenyl)methanone (8d)

Colour: yellow semisolid. Yield: 81%. IR (KBr, ν, cm^{-1}): 1678 (C=O), 1606(aromatic C=C), 3028, 2926 and 2853cm^{-1} was due to C-H stretch of aromatic and SCH_3 group. ^{1}H NMR (CDC_{l3}-400MHz) δ ppm: 2.55(s, 3H, SCH_3), 6.7-7.8(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100

MHz) δ ppm: 14.8, 115.3, 126.4, 129.8, 130.7, 133.2, 134.4, 143, 166.4, 194.4. MS (ESI, m/z): 246 (M+). Anal. Calcd. for $C_{14}H_{11}FOS$: C; 68.27, H; 4.50, O; 6.50, S; 13.02, Found: C; 68.26, H; 4.51%.

(4-Methoxyphenyl)(3- (methylthio)phenyl) methanone (8e)

Colour: light yellow semisolid. Yield: 79%. IR (KBr, ν, cm^{-1}): 1680 (C=O), 1608(aromatic C=C), 3028, 2922 and 2881cm^{-1} was due to C-H stretch of aromatic, SCH_3 and –OCH_3 groups. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 7.07-7.62(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.7, 55.6, 114, 126.4, 130.7, 131.2, 134.3, 143.2, 164.2, 194.2. MS (ESI, m/z): 258 (M^+). Anal. Calcd. for $C_{15}H_{14}O_2S$: C; 69.74, H; 5.46, O; 12.39, S; 12.41, Found: C; 69.72, H; 5.44%.

Cyclohexyl(3- (methylthio)phenyl) methanone (8f)

Colour: orange semisolid. Yield: 83%. IR (KBr, ν, cm^{-1}): 1682 (C=O), 1591(aromatic C=C), 3025, 2920 and 2854cm^{-1} was due to C-H stretching. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 1.49-2.93(m, 11H, Cyclohexyl group), 7.41-7.77(m, 4H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.6, 25.3, 25.8, 25.7, 126.5, 132.7, 132.2, 133.2, 202.02. MS (ESI, m/z): 234 (M+). Anal. Calcd. for $C_{14}H_{18}OS$: C; 71.75, H; 7.74, O; 6.83, Found: C; 71.75, H; 7.73%.

2.2.2. General procedure for the synthesis of itaconic acids (10a-f)

Potassium tertiary butoxide is used as a base for stobbe condensation. It was obtained by the reaction of tertiary butyl alcohol (50ml) with potassium (1.0908g, 0.0219mole) under dry nitrogen gas atmosphere at reflux temperature for an hour. Substituted benzophenones **9a-f** (5g, 0.0219mole) was added quickly and continued heating for about half an hour to dissolve the ketone. To this freshly distilled diethyl succinate (3.814g, 0.0219mole) was added and refluxed for twelve hour. The cooled mixture was neutralized by adding 5N HCl (30ml), was concentrated to 50ml by distillation and diluted with water (50ml). The cis and trans isomeric forms of itaconic acid half esters **10a-f** were extracted into ether (3x25ml) and then into saturated aqueous sodium bicarbonate solution which was acidified with cold concentrated HCl. It was recrystallised from ethanol gave a solid compounds with good yield. The itaconic acid half esters were saponified by refluxing in methanol (30ml) and water (30ml) mixture containing sodium hydroxide (3g) and acidified with concentrated hydrochloric acid to give solid compounds. They were purified by recrystallisation from ethanol gave **10a-f** in good yields.

2-((3-Methylthio)phenyl) methylene)succinic acid (10a)

Colour: white crystalline solid. Yield: 84%. m.p. 105-108°C. IR (KBr, ν, cm^{-1}): 3398-3200 (Carboxylic -OH group), 1702(-CH_2CO), 1681(α-β-unsaturated C=O), 1611-1607(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 3.58(s, 2H, CH_2), 10.98(bs, 2H, COOH), 6.9-7.4(m, 9H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.7, 33.3, 115.3, 122.6, 124.8, 125.7, 127.8, 128.5, 130.7, 132.2, 139.2, 140.0, 141.4, 171.2, 174.5.

MS (ESI, m/z): 328 (M^+). Anal. Calcd. for $C_{18}H_{16}O_4S$: C; 65.84, H; 4.91, O; 19.49, S; 9.76, Found: C; 65.83, H; 4.92%.

2-((3-Methylthio)phenyl)(p-tolyl)methylene)succinic acid (10b)

Colour: white crystalline solid. Yield: 81%. m.p. 91-93°C. IR (KBr, ν, cm^{-1}): 3589-3275(carboxylic OH), 1694(-CH_2CO), 1684 (α-β-unsaturated C=O), 1606(aromatic C=C), 1611-1605(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.33(s, 3H, -CH_3 group), 9.7-9.9(bs, 2H, -COOH), 6.89-7.25(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 21.3, 115.1, 122.6, 124.2, 125.7, 130.6, 130.7, 134.2, 137.6, 138.2, 140.0, 160.5, 171.4, 174.2. MS (ESI, m/z): 343 (M^+). Anal. Calcd. for $C_{19}H_{18}O_4S$: C; 66.65, H; 5.29, O; 18.68, S; 9.37 Found: C; 66.63, H; 5.28%.

2-((3-Methylthio)phenyl)(4-nitrophenyl)methylene)succinic acid (10c)

Colour: pale yellow solid. Yield: 79%. m.p. 98-101°C. IR (KBr, ν, cm^{-1}): 3599-3279(carboxylic OH), 1692(-CH_2CO), 1682 (α-β-unsaturated C=O), 1601(aromatic C=C), 1612-1609(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 9.8-10.1(s, 2H, -COOH), 6.8-8.21(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.7, 33.4, 115.1, 122.6, 123.8, 124.4, 125.6, 127.6, 130.5, 139.2, 140.0, 147.2, 160.6, 171.3, 174.1. MS (ESI, m/z): 374 (M^+). Anal. Calcd. for $C_{18}H_{18}NO_6S$: C; 57.90, H; 4.05, O; 25.71, S; 8.59, N; 3.75 Found: C; 56.89, H; 4.03%.

2-((3-Methylthio)phenyl)(4-flourophenyl)methylene)succinic acid(10d)

Colour: yellow solid. Yield: 78%. m.p. 97-100°C. IR (KBr, ν, cm^{-1}): 3598-3270(carboxylic OH), 1691(-CH_2CO), 1681 (α-β-unsaturated C=O), 1602(aromatic C=C), 1611-1606(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 9.7-10.2(bs, 2H, -COOH), 6.9-7.36(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 33.5, 115.2, 122.4, 123.8, 124.5, 125.5, 130.6, 131.3, 137.4, 139.2, 140.0, 147.2, 160.7, 162.3, 171.3, 174.2. MS (ESI, m/z): 346 (M^+). Anal. Calcd. for $C_{18}H_{15}FO_4S$: C; 62.42, H; 4.36, O; 18.48, S; 9.26, F; 5.48 Found: C; 62.42, H; 4.35%.

2-((3-Methylthio)phenyl)(4-flourophenyl)methylene)succinic acid (10e)

Colour: white solid. Yield: 82%. m.p. 102-105°C. IR (KBr, ν, cm^{-1}): 3589-3258(carboxylic OH), 1691(-CH_2CO), 1679 (α-β-unsaturated C=O), 1599 (aromatic C=C), 1610-1602(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 9.2-9.9(bs, 2H, -COOH), 6.8-7.3(m, 8H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.7, 55.6, 114.1, 115.2, 122.5, 124.8,

125.5, 130.6, 133.2, 137.4, 139.2, 140.0, 159.2, 160.7, 171.3, 174.2. MS (ESI, m/z): 358 (M^+). Anal. Calcd. for $C_{19}H_{18}O_5S$: C; 63.67, H; 5.06, O; 22.32, S; 8.95, Found: C; 63.65, H; 5.05%.

2-(Cyclohexyl(3-methylthio)phenyl)methylene)succinic acid (10f)

Colour: pale yellow solid. Yield: 77%. m.p. 98-102^oC. IR (KBr, ν, cm^{-1}): 3595-3265(carboxylic OH), 1692(-CH_2CO), 1680 (α-β-unsaturated C=O), 1598 (aromatic C=C), 1609-1599(conjugated C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 1.43-1.69(m, 11H, cyclohexyl group), δ 7.42-7.69(m, 4H, Ar-H). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 24.2, 25.3, 26.2, 32.3, 84.6, 117.4, 122.1, 124.2, 125.9, 130.5, 139.5, 140.0, 155.2, 171.3, 174.2. MS (ESI, m/z): 334 (M^+). Anal. Calcd. for $C_{18}H_{22}O_4S$: C; 64.65, H; 6.63, O; 19.14, S; 9.59, Found: C; 64.63, H; 6.61%.

2.2.3. General procedure for the synthesis of benzhydryl succinic acids (11a-f)

The **11a-f** compounds were obtained from itaconic acids **10a-f** (5g, 0.01524mole) which were dissolved in 5% sodium hydroxide and cooled to 5-6^OC and then powdered 5% sodium- amalgam was added slowly and stirred over night, on usual work up, the products **11a-f** were obtained as white crystalline solid in good yields.

2-((3-Methylthio)phenyl)(phenyl)succinic acid (11a)

Colour: white crystalline solid. Yield: 81%. m.p. 71-73^oC. IR (KBr, ν, cm^{-1}): 3400-3150(carboxylic OH), 1712(carbonyl C=O), 1585 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.5(d, J=6Hz, 2H, C_c-H), 3.4-3.6(q, 1H, C_b-H), 3.7(d, J=7Hz, 1H, C_a-H), 6.7-7.3(m, 9H Ar-H), 9.9(bs, 2H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 34.5, 46.1, 47.3, 123.1, 124.5, 125.9, 128.2, 129.4, 139.3, 140.1, 143.2, 177.2, 178.2. MS (ESI, m/z): 330 (M^+). Anal. Calcd. for $C_{18}H_{18}O_4S$: C, 65.43; H, 5.49; O, 19.37; S, 9.70, Found: C; 65.42, H; 5.47.

2-((3-(Methylthio)phenyl)(p-tolyl)methyl)succinic acid (11b)

Colour: pale pink solid. Yield: 79%. m.p. 62-65^oC. IR (KBr, ν, cm^{-1}): 3395-3170(carboxylic OH), 1714(carbonyl C=O), 1589 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 2.4(d, J=6Hz, 2H, C_c-H), 3.5-3.7(q, 1H, C_b-H), 3.8(d, J=7Hz, 1H, C_a-H), 2.35 (s, 3H CH_3), 6.1-7.3(m, 8H Ar-H), 9.7(bs, 2H, COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 21.2, 34.6, 46.2, 47.2, 123.2, 124.4, 126.7, 128.0, 129.3, 135.5, 137.2, 139.3, 143.1, 177.3, 178.1. MS (ESI, m/z): 334 (M^+). Anal. Calcd. for $C_{19}H_{20}O_4S$: C, 66.26; H, 5.85; O, 18.58; S, 9.31, Found: C; 66.24, H; 5.86%.

2-((3-(Methylthio)phenyl)(4-nitrophenyl)methyl)succinic acid(11c)

Colour: pink solid. Yield: 82%. m.p. 67-69^oC. IR (KBr, ν, cm^{-1}): 3396-3172(carboxylic OH), 1718(carbonyl C=O), 1592 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 2.6(d, J=6Hz, 2H, C_c-H), 3.3-3.5(q, 1H, C_b-H), 3.9(d, J=7Hz, 1H, C_a-H), 7.2-8.1(m, 8H Ar-H), 10.8(bs, 2H, COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.7, 34.5, 46.3, 47.1, 123.2, 124.5, 126.6, 129.3, 139.2, 143.4, 145.4, 146.2, 177.3, 178.2. MS (ESI, m/z): 375 (M^+). Anal. Calcd. for $C_{18}H_{17}NO_6S$: C, 57.59; H, 4.56; N, 3.73; O, 25.57; S, 8.54, Found: C; 57.58, H; 4.55%.

2-((4-Fluorophenyl)(3-(methylthio)phenyl)methyl)succinic acid (11d)

Colour: pale yellow solid. Yield: 78%. m.p. 75-77^oC. IR (KBr, ν, cm^{-1}): 3399-3174(carboxylic OH), 1720(carbonyl C=O), 1598 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.5-2.7(d, J=6Hz, 2H, C_c-H), 3.3-3.4(q, 1H, C_b-H), 4.2(d, J=7Hz, 1H, C_a-H), 7.2-7.5(m, 8H Ar-H), 10.7(bs, 2H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 34.6, 46.5, 47.2, 116.3, 123.4, 124.3, 126.7, 129.8, 135.4, 139.1, 143.3, 160.3, 177.2, 178.3. MS (ESI, m/z): 348 (M^+). Anal. Calcd. for $C_{18}H_{17}FO_4S$: C, 62.06; H, 4.92; F, 5.45; O, 18.37; S, 9.20, Found: C; 61.98, H; 4.94%.

2-((4-Methoxyphenyl)(3-(methylthio)phenyl)methyl)succinic acid (11e)

Colour: yellow solid. Yield: 82%. m.p. 80-82^oC. IR (KBr, ν, cm^{-1}): 3397-3175(carboxylic OH), 1717(carbonyl C=O), 1595 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 2.5-2.8(d, J=6Hz, 2H, C_c-H), 3.3-3.4(q, 1H, C_b-H), 4.4(d, J=7Hz, 1H, C_a-H), 6.9-7.3(m, 8H Ar-H), 10.8(bs, 2H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 55.7, 34.5, 46.2, 47.3, 114.7, 123.3, 124.2, 126.5, 129.6, 132.5, 139.4, 143.1, 158.3, 177.3, 178.1. MS (ESI, m/z): 360 (M^+). Anal. Calcd. for $C_{19}H_{20}O_5S$: C, 63.32; H, 5.59; O, 22.20; S, 8.90, Found: C; 63.33, H; 5.5%.

2-(Cyclohexyl(3-(methylthio)phenyl)methyl)succinic acid (11f)

Colour: pale yellow solid. Yield: 85%. m.p. 63-65^oC. IR (KBr, ν, cm^{-1}): 3399-3178(carboxylic OH), 1720(carbonyl C=O), 1597 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.5-2.7(d, J=6Hz, 2H, C_c-H), 3.0(q, 1H, C_b-H), 3.0(d, J=7Hz, 1H, C_a-H), 1.4-1.8(m, 11H cyclohexyl group), 7.2-7.5(m, 3H Ar-H), 10.9(bs, 2H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) δ ppm: 14.8, 26.1, 34.5, 46.2, 47.3, 114.7, 123.3, 124.2, 126.5, 129.6, 132.5, 139.4, 143.1, 158.3, 177.3, 178.1. MS (ESI, m/z): 360 (M^+). Anal. Calcd. for $C_{18}H_{24}O_4S$: C, 64.26; H, 7.19; O, 19.02; S, 9.53, Found: C; 64.24, H; 7.20%.

2.2.4. General procedure for the synthesis of the tetralone acids (13a-f)

A solution of **11a-f** in acetyl chloride (20ml) was refluxed for 2h. The excess acetyl chloride was removed by distillation and the residue after dissolving in benzene (50ml) was repeatedly washed with cold 5% sodium bicarbonate solution (2x20ml) to remove the acidic

compounds and then with cold water. The organic layer after drying over anhyd.Na_2SO_4 was concentrated to give a brown semisolid anhydride **12a-f** in good yields. The IR peaks appeared at 1780 and 1784 indicated the formation of cyclic anhydride.

A solution of anhydride **12a-f** (5g, 0.0160mole) in dry dichlomethane (50ml) was added over a period of 15min to a magnetically stirred solution of anhyd. $AlCl_3$ (2.133g, 0.0160mole) in dry dichloromethane (50ml) at 0°C. The reaction mixture was further stirred at 0°C for 7h. The reaction mixture was treated with cold 5N hydrochloric acid. The organic layer was separated by extracting into chloroform (2x50ml) and washed thoroughly with cold 5N HCl (2x30ml) and then water (2x40ml). The acidic compound was extracted into the saturated bicarbonate solution (3x40ml) and acidified with concentrated hydrochloric acid (50ml) and crushed ice (200g) to give a solid compounds in good yields.

7-(Methylthio)-4-oxo-1-phenyl-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13a)

Colour: yellow crystalline solid. Yield: 81%. m.p: 92-95°C. IR (KBr, ν, cm^{-1}): 3500-3699(OH of COOH), 1724(C=O of COOH), 1662 (tetralone C=O), 1585(aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.8(dd, J=7Hz, 2H, C_2-H), 3.6-3.5(q, 1H, C_3-H), 4.3(d, J=7Hz, 1H, C_4-H), 7.2-7.7(m, 8H Ar-H), 10.8(bs, 1H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.8, 37.5, 43.4, 45.7, 123.1, 124.3, 126.4, 128.5, 129.2, 130.3, 140.4, 143.1, 178.1, 196.9. MS (ESI, m/z): 312 (M^+). Anal. Calcd. for $C_{18}H_{16}O_3S$: C, 69.21; H, 5.16; O, 15.37; S, 10.26; Found: C, 69.22; H, 5.15%.

7-(Methylthio)-4-oxo-1-(p-tolyl)-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13b)

Colour: pale yellow crystalline solid. Yield: 76%. m.p: 112-115°C.IR (KBr, ν, cm^{-1}): 3500-3703(OH of COOH), 1714(C=O of COOH), 1681 (tetralone C=O), 1696 (aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 2.3(s, 3H,CH_3), 2.9(dd, J=7Hz, 2H, C_2-H), 3.4-3.5(q, 1H, C_3-H), 4.3(d, J=7Hz, 1H, C_4-H), 7.3-7.7(m, 7H Ar-H), 10.9(bs, 1H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.7, 21.2, 37.3, 41.7, 43.4, 123.1, 124.2, 128.3, 129.2, 130.3, 135.1, 143.5, 178.2, 196.8. MS (ESI, m/z): 326 (M^+). Anal. Calcd. for $C_{19}H_{18}O_3S$ C, 69.91; H, 5.56; O, 14.70; S, 9.82; Found: C, 69.92; H, 5.55%.

7-(Methylthio)-1-(4-nitrophenyl)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13c)

Colour: yellow crystalline solid. Yield: 64%. m.p: 97-103°C. IR (KBr, ν, cm^{-1}): 3500-3300(OH of COOH), 1716(C=O of COOH), 1683 (tetralone C=O), 1697(aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.9(dd, J=7Hz, 2H, C_2-H), 3.6-3.7(q, 1H, C_3-H), 4.3(d, J=7Hz, 1H, C_4-H), 7.2-8.0(m, 7H Ar-H), 10.9(bs, 1H COOH).
^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.8, 37.3, 43.7, 45.4, 123.2, 124.4, 129.2, 130.5, 135.1, 140.3, 143.5, 145.3, 146.3, 178.4, 196.7. MS (ESI, m/z): 357 (M^+). Anal. Calcd. for $C_{18}H_{15}NO_5S$: C, 60.49; H, 4.23; N, 3.92; O, 22.38; S, 8.97; Found: C, 60.50; H, 4.22.

1-(4-Fluorophenyl)-7-(methylthio)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13d)

Colour: orange solid. Yield: 73%. m.p. 73-75°C. IR (KBr, ν, cm^{-1}): 3500-3300(OH of COOH), 1716(C=O of COOH), 1680 (tetralone C=O), 1606(aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.52(s, 3H, SCH_3), 2.8-2.9(t, J=7Hz, 2H, C_2-H), 3.6-3.5(q, 1H, C_3-H), 4.3(d, J=7Hz, 1H, C_4-H), 7.2-8.2(m, 7H Ar-H), 10.16(bs, 1H, COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.7, 37.2, 43.6, 45.3, 116.2, 123.3, 124.5, 129.4, 130.3, 135.5, 140.7, 143.5, 160.3, 178.3, 196.8. MS (ESI, m/z): 330 (M^+). Anal. Calcd. for $C_{18}H_{15}FO_3S$: C, 65.44; H, 4.58; F, 5.75; O, 14.53; S, 9.71; Found: C, 65.43; H, 4.56%.

1-(4-Methoxyphenyl)-7-(methylthio)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13e)

Colour: orange crystalline solid. Yield: 68%. m.p. 109-112°C. IR (KBr, ν, cm^{-1}): 3500-3300(OH of COOH), 1721(C=O of COOH), 1683 (tetralone C=O), 1610(aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 3.8(s, 3H OCH_3) 2.6-2.8(d, J=7Hz, 2H, C_2-H), 3.6(q, 1H, C_3-H), 4.4(d, J=7Hz, 1H, C_4-H), 7.1-7.8(m, 7H Ar-H), 10.8(bs, 1H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.8, 37.3, 43.7, 45.5, 55.8, 114.7, 123.2, 124.6, 129.3, 130.4, 132.6, 140.6, 143.6, 158.3, 178.4, 196.9. MS (ESI, m/z): 342(M^+). Anal. Calcd. for $C_{19}H_{18}O_4S$: C, 66.65; H, 5.30; O, 18.69; S, 9.36; Found: C, 66.66; H, 5.29%.

1-Cyclohexyl-7-(methylthio)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (13f)

Colour: pale pink solid. Yield: 71%. m.p: 97-101°C. IR (KBr, ν, cm^{-1}): 3500-3300(OH of COOH), 1723(C=O of COOH), 1685 (tetralone C=O), 1596(aromatic C=C) cm^{-1}. ^1H NMR ($CDCl_3$-400MHz) δ ppm: 2.53(s, 3H, SCH_3), 2.6-2.9(dd, J=7Hz, 2H, C_2-H), 3.3(q, 1H, C_3-H), 3.1(d, J=7Hz, 1H, C_4-H), 1.3-1.8(m. 11H, cyclohexyl), 7.3-7.8(m, 3H, Ar-H), 9.6(bs, 1H COOH). ^{13}C NMR ($CDCl_3$ -100 MHz) ppm: 14.8, 26.1, 31.0, 36.1, 37.0, 38.1, 39.2, 123.3, 128.4, 138.7, 143.1, 178.1, 196.4. MS (ESI, m/z): 318(M^+). Anal. Calcd. for $C_{18}H_{22}O_3S$: C, 67.89; H, 6.96; O, 15.07; S, 10.07; Found: C, 67.88; H, 6.97%.

2.3. Antimitotic assay

The synthesized new tetralone acids (**13a-f**) were screened for antimitotic activity by onion root tip method and their ID_{50} values were determined. The materials required are aceto-orcein 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 ppm). To study the effect of tetralone acid intermediates on somatic cells, onion base was immersed to an extent of about half a centimeter in a control solution tube and the sample solution tube containing compounds (ca 7x3 cm), after removing the old roots from it and immersion is continued for 24hrs.,

for germination. After 24hrs, 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 24hrs. Carney's solution II was decanted carefully and the root tips were washed with preserving solvent (70 % ethanol). The fixed root tips were persevered in 70% ethanol in th refrigerator. The root tips were taken in the watch glass and stained with a drop of aceto-orcein stain and a drop of 1N HCl (7:1). The glasses were warmed and kept for 1hr. The roots were taken on a clean glass slide and squashed using 45% acetic acid following the method of Levan.[19] 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. Mitotic Index (MI) was calculated by Fissceja[20] method. The mitotic index was determined by examination of minimum zone inhibition of cells. Three replicates were made for each calculation. The slides were observed under microscope and photographed.

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

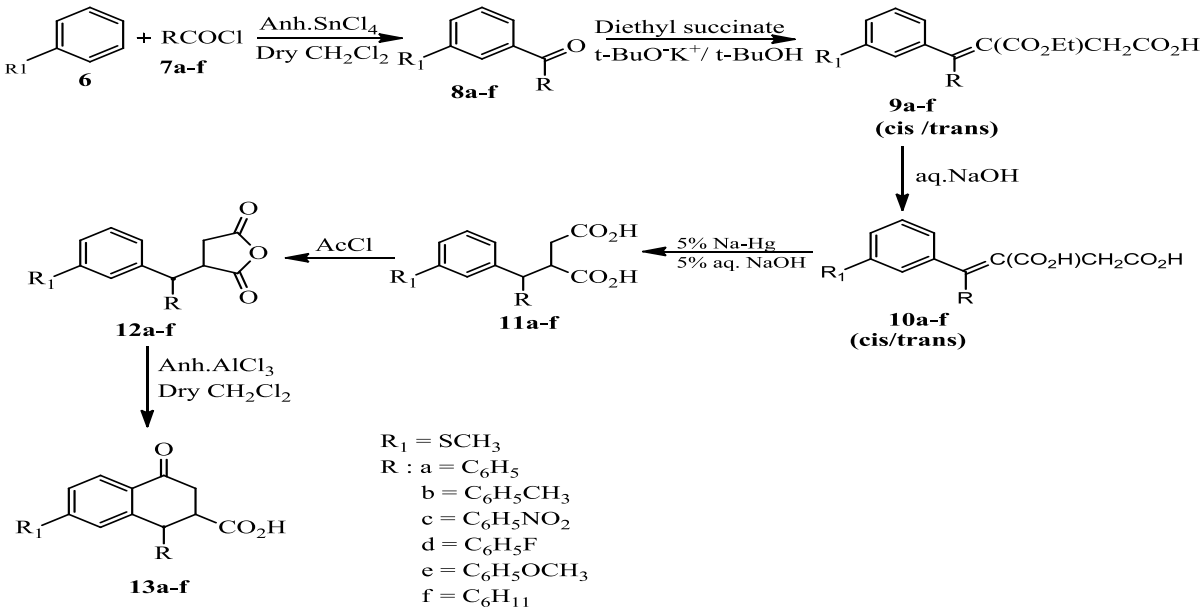
The percentage of the number of dividing cells compared to the control and the percent inhibition of mitosis by antimitotic agent at a different concentration such as 100, 200 and 400ppm against a control were calculated. The concentration needed for 50% inhibition (ID_{50}) was extrapolated from the graph by plotting concentration verses percentage of inhibition. The ID_{50} values for new tetralone acid intermediates of analogues of podophyllotoxin for their antimitotic activity were calculated individually following hakala[21] method.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of tetralone acid intermediates (**13a-f**) has been carried out using Gensler's method with slight changes in reagents and experimental procedure (**Scheme -1**). The starting material acid chlorides (**7a-f**) were prepared by the refluxion with thionyl chloride in dry benzene.[22] The substituted benzophenones (**8a-f**) were obtained by Freidel- Craft's acylation of thioanisole (**6**) with substituted benzoyl chloride (**7a-f**) in presence of stannic in dry dichloromethane. The compounds (**10a-f**) were prepared by stobbe condensation of **(8a-f**) with diethyl succinate in presence of potassium t-butoxide as the base[23] in ter.butanol. The stobbe condensation reaction occurred smoothly at reflux temperature and were obtained as a mixture of cis and trans isomers in good yields. The saponification of itaconic acid half esters (**9a-f**) were carried out by refluxing with sodium hydroxide in water-methanol mixture to form itaconic acids (**10a-f**) in cis and trans isomeric forms. The benzhydryl succinic acids (**11a-f**) were prepared by the catalytic hydrogenation of compounds (**10a-f**) in the presence of sodium-amalgam in aqueous sodium hydroxide.[24]

The compounds (**11a-f**) were converted into benzhydryl succinic anhydrides (**12a-f**) by refluxtion with acetyl chloride. The tetralone acids (**13a-f**) were prepared by the intramolcular Friedel-Craft's acylation reaction of benzhydryl succinic anhydrides in the presence of Lewis acid anhyd. aluminium chloride in dichloromethane.[25] The products were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis data.



Scheme-1

3.2. Antimitotic activity

Allium cepa has been used to evaluate the antimitotic activity of newly synthesised tetralone acid intermediates of analogues of podophyllotoxin by onion root tip method. The antimitotic activity of tetralone acid intermediates were shown in the **table-1**. The presence of different substituent's on the ring C causes the changes in activity. The results showed that the percentage of inhibition of compounds **13e** and **13f** was significantly highest with 400 ppm for 24hrs., compound **13a** and **13c** showed least inhibition while **13b** and **13d** exhibited moderate inhibition of abnormal when compared to control. The compound **13e** containing electron substituent, methoxy group on ring C, which is accounted for the enhanced antimitotic activity than the control. From the obtained results, it is clearly indicated that the substituent played in exhibiting major role antimitotic activity on ring C. It is evident that the novel tetralone acids showed moderate to good antimitotic activity.

Table-1: Antimitotic activity of the compounds (13a-f) by onion root tip method.

Comp. no.	Conc. in ppm	% dividing cells	% dividing cells compared to control	% inhibition Compared to control	ID_{50} in ppm
13a	100	20.85	57.91	42.08	250
	200	18.96	52.66	47.34	
	400	14.72	40.88	59.11	
13b	100	19.55	54.30	45.69	170
	200	16.29	45.25	54.75	
	400	13.55	37.63	62.36	
13c	100	21.90	60.83	39.16	260
	200	19.62	54.50	45.50	
	400	15.03	41.75	58.25	
13d	100	19.52	54.22	45.78	130
	200	16.41	45.58	54.41	
	400	12.91	35.86	64.13	
13e	100	16.84	46.77	53.22	85
	200	15.31	42.52	57.47	
	300	12.92	35.88	64.11	
13f	100	16.83	46.75	53.25	105
	200	15.35	42.63	57.36	
	400	10.28	28.55	71.44	
control	-	36.00	100.00	00.00	-

4. CONCLUTION

This method is a very efficient method for the synthesis of tetralone acid intermediates. The new tetralone acids (**13a-f**) were synthesized in six steps. The tetralone acids (13a-f) were synthesised in good yields using less expensive and readily available chemicals. The substituted benzophenones were easily converted into itaconic acids by stobbe condensation followed by saponification. The double bond of the itaconic acids were reduced to benzhydryl succinic acids by catalytic hydrogenation in autoclave. The structures of the synthesized compounds were confirmed by analytical and spectral data. All the synthesized tetralone acids (**13a-f**) were screened for their antimitotic activity. Among the synthesized tetralone acids, compound **13e** and **13f** showed good antimitotic activity. The compounds **13b** and **13d** showed moderate activity and compounds **13a** and **13c** exhibited least antimitotic activity.

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