Synthesis of new substituted tetralone acids and evaluation of antimitotic activity
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1. INTRODUCTION

Podophyllotoxin [1] (Figure 1) and its several analogues are of current interest due to their use in cancer chemotherapy [2-4]. Podophyllotoxin isolated from two important medicinal plants named podophyllum emodi an Indian species and podophyllum peltatum, a North American species [5]. It also occurs in many other plants of podophyllum species. It belongs to the family of natural product called lignan. Podophyllotoxin showed other biological activities such as cathartic, antitropical skin disease, antimalarial, anti-HIV (AIDS) etc. [6].

In view of the above facts, it was decided to modify the structure of podophyllotoxin and synthesized tetralone acids [7-10]. They were synthesized by replacing methylene dioxy group in podophyllotoxin with fluoro and methoxy group, lactone ring with carboxylic acid and tri methoxy phenyl group with substituted phenyl to study the structure activity relationship. The analogues of podophyllotoxin were synthesized using Gensler's method [11] with some changes in reagents and experimental procedure. The synthesized tetralone acids were screened for their antimitotic activity by onion root method [12].

2. EXPERIMENTAL

2.1. Materials and methods

All the chemicals 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. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded using tetramethyl silane (TMS) as an internal reference on Bruker spectrometers; 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 preparation of substituted (3-fluoro-4-methoxyphenyl)(phenyl)methanone (2a-d)

2-fluoro anisole (10g, 0.079 mol) and anhyd. aluminum chloride (11.6 g, 0.0872 mol) were taken in dichloromethane (50 mL). The reaction mixture was cooled to 0-5 °C. A solution of substituted benoyl chloride (1a-d) (14.87g, 0.087mol) in dichloromethane (20 mL) was added slowly over a period of 1hr. The reaction mixture was stirred for 12 hr at room temperature. Completion of the reaction was known by TLC. The mixture was cooled to 0-5 °C and stirred with con. HCl (50mL). The reaction mixture was stirred at room temperature for 2 hr. The product in dichloromethane layer was separated and washed with 5 %aq. sodium bicarbonate solution (2x20 mL) and then with water (2x20 mL). The solvent was removed by distillation. The product was
recrystallized from diethyl ether to afford substituted (3-fluoro-4-methoxyphenyl)(phenyl)methanone in good yields.

(3-fluoro-4-methoxyphenyl)(4-methoxyphenyl)methanone (2a): Color: white solid. Yield: 67.7%. M.P: 109-112°C. IR (KBr,v,Cm-1): 1690 (C=O), 1592 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 3.7 (s, 6H, -OCH3), 6.8-7.2 (m, 7H, Ar-H); 13C NMR (CDCl3-100 MHz) δ ppm: 55.8, 115, 118, 127, 137.7, 152.3,155.2, 185.3, 198.4. MS (ESI, m/z): 260.9 (M+). Anal. Calcd. for C15H13O3: C, 72.34; H, 5.01 %.

(3-fluoro-4-methoxyphenyl)(p-tolyl)methanone (2b): Color: white solid. Yield: 73%. M.P: 91-93°C. IR (KBr,v,Cm-1): 1697 (C=O), 1598 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 3.9 (s, 3H, -CH3), 3.6 (s, 3H, -OCH3), 6.9-7.7 (m, 7H, Ar-H); 13C NMR (CDCl3-100 MHz) δ ppm: 54.8, 116.8, 117, 123.7, 129, 132.4, 134, 140.3, 154.2, 156.2, 197.2. MS (ESI, m/z): 231.9 (M+). Anal. Calcd. for C16H15O2: C, 73.08; H, 4.79 %.

(3-fluoro-4-methoxyphenyl)(p-tolyl)methanone (2c): Color: yellow solid. Yield: 75%. M.P: 123-125 °C. IR (KBr,v,Cm-1): 1701 (C=O), 1599 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 2.2 (s, 3H, -CH3), 3.6 (s, 3H, -OCH3), 6.9-7.7 (m, 7H, Ar-H); 13C NMR (CDCl3-100 MHz) δ ppm: 27.2, 56.8, 116.7, 117.2, 127.3, 129.3, 132.2, 133.2, 137.4, 147.1, 152.3, 154.2, 197.0. MS (ESI, m/z): 245.03. Anal. Calcd. for C16H15O2: C, 73.76; H, 5.36. Found: C, 73.72; H, 5.35 %.

(3,4-dimethoxyphenyl)(3-fluoro-4-methoxyphenyl)methanone (2d): Color: Yellow color gummy mass. Yield: 73%. IR (KBr,v,Cm-1): 1696 (C=O), 1589 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 3.9 (s, 9H, -OCH3), 7.2-7.7 (m, 6H, Ar-H); 13C NMR (CDCl3-100 MHz) δ ppm:58.3, 58.8, 58.9, 117.3, 117.9, 119, 121.2, 124.3, 126.7, 133.8, 134.2, 149.5, 152.3, 157.3, 158.9, 198.7. MS (ESI, m/z): 291.9. Anal. Calcd. for C19H15O4: C, 66.2; H, 5.21 . Found: C, 65.08; H, 5.17 %.

2.2. General procedure for the synthesis of substituted 2-((3-fluoro-4-methoxyphenyl)(phenyl)methylene)succinic acid

Sodium hydride (1.84g, 0.0767 mol) was added in portions to the stirred suspensions of dry diethyl succinate (20.02g, 0.115 mol) and substituted (3-fluoro-4-methoxyphenyl)(phenyl)methanone (2a-d) (10g, 0.0383mol) in dry Benzene (30 mL) under nitrogen gas atmosphere. Absolute ethanol (8.8g, 0.192 mol) was added slowly over a period of 30min. during the addition of ethanol, evolution of hydrogen gas observed. The reaction mass was stirred at 25-30 °C for 15hr under nitrogen. The completion of the reaction was confirmed by TLC. The mixture was cooled and acidified by the addition of dilute glacial acetic acid, compound was extracted into ether and then into saturated sodium carbonate solution. The sodium carbonate extract was neutralized by dil acetic acid. The precipitated gummy mass was extracted into dichloromethane. The organic layer was washed with water and concentrated under vacuum to afford brown gummy mass (3a-d). The substituted succinic half esters (3a-d) (7g, 0.0114 mol) were taken in methanol (28ml) and water (28ml) mixture containing NaOH (3.6g, 0.090mol). Reaction mass was refluxed for 3-4hr, distilled reaction mass completely to obtain the residue and diluted with 30ml of water. The aqueous layer was washed with ether and then neutralized with dil HCl. The compound extracted into dichloromethane and organic layer dried over anhydrous sodium sulphate and solvent was removed by distillation to afford yellow color residue. Purification of the residue done by column chromatography on silica gel (EtoAc/Cyclohexane, 8:3) gave slight yellow gummy mass.

2-((3-fluoro-4-methoxyphenyl)(4-methoxyphenyl)methylene)succinic acid (4a): Color: light yellow gummy mass. Yield: 64%. IR (KBr,v,Cm-1): 3600-3300(Carboxylic -OH), 1700 (-CH2- C=O), 1685 (α, β, unsaturated C=C), 1593 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 3.1 (s, 2H, -CH2), 3.74 (s, 6H, -OCH3), 6.7-7.7 (m, 7H, Ar-H), 10.7 (bs, 2H, -COOH); 13C NMR (CDCl3-100 MHz) δ ppm: 33.7, 56.2, 112.3, 115.7, 119.2, 117.4, 127.8, 133.3, 134.3, 148.2, 153.8, 160.3, 166.2,173.3. MS (ESI, m/z): 361.3. Anal. Calcd. for C19H15O4: C, 63.33; H, 4.76 . Found: C, 63.28; H, 4.73 %.

2-((3-fluoro-4-methoxyphenyl)(phenyl)methylene) succinic acid (4b): Color: light yellow color gummy mass. Yield: 66%. IR (KBr,v,Cm-1): 3590-3270(Carboxylic -OH), 1695 (-CH2- C=O), 1681 (α, β, unsaturated C=C), 1598 (Aromatic C=C); 1H NMR (CDCl3-400MHz) δ ppm: 2.8 (s, 2H, -CH2), 3.73 (s, 3H, -OCH3), 6.8-7.3 (m, 8H, Ar-H), 11.2 (bs, 2H, -COOH); 13C NMR (CDCl3-100 MHz) δ ppm:38.2, 117, 117.4, 126.2, 128, 128.7, 134.9, 141.2, 148.3, 152.7, 154.7, 172.3, 173.7. MS (ESI, m/z): 331.9. Anal. Calcd. for C19H15O4: C, 65.45; H, 4.58 . Found: C, 65.39; H, 4.57 %.

2-((3-fluoro-4-methoxyphenyl)(p-tolyl)methylene) succinic acid (4c): Color: light yellow gummy mass. Yield: 69%. IR (KBr,v,Cm-1): 3510-3272(Carboxylic -OH), 1593(Conjugated C=C), 1411 - 1413 cm-1 (Unsaturated C=C).
1696 (–CH=–C=O), 1687 (α, β, unsaturated C=O), 1604 (Aromatic C=C); 1H NMR (CDCl3-400MH) δ ppm: 2.7 (s, 3H, –CH3), 3.1 (s, 2H, –CH2), 3.92 (s, 3H, –OCH3), 6.6-7.09 (m, 7H, Ar-H); 13C NMR (CDCl3-100 MH) δ ppm: 22.3, 56.3, 71.4, 117.4, 117.9, 112.4, 124.38, 126.8, 129.4, 134.5, 137.9, 137.3, 144.4, 155.7, 162.2, 172.2, 173.2, 178.4, 179.3. MS (ESI, m/z): 362.9.

2,4-Dimethylphenyl-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (5a–d)

In an autoclave vessel, the compound (4a–d) (5 g, 0.011 mol) was added dropwise to the stirred suspensions of anhydride aluminum chloride (2.9 g, 0.0117 mol) in dichloromethane (30 mL) at 0-5°C under inert atmosphere. The reaction mixture was stirred for 6h at 25-30°C. The completion of the reaction was known by TLC. Excess of acetyl chloride was concentrated out to afford a benzhydryl anhydride as gummy mass (6a–d) in good yields.

Compounds (5a–d) (0.011 mol) and acetyl chloride (50 mL) was refluxed at 45-50°C for 6hr. Completion of the reaction was known by TLC. Excess of acetyl chloride was concentrated atmospherically at 45-50°C. The resultant mixture was dissolved in dichloromethane 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 benzhydryl anhydride as gummy mass (6a–d) in good yields.

Above compounds (6a–d) (3 g, 0.0087 mol) were dissolved in dichloromethane (30 mL) and added dropwise to the stirred suspensions of anhydride aluminum chloride (2.9 g, 0.00217 mol) in dichloromethane (30 mL) at 0-5°C under inert 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 Na2CO3 solution. The sodium carbonate extracts were
acidified by dil. HCl to give products in good yields. Yellow solid was purified by column chromatography on silica gel (EtOAc/n-hexane/MeOH) gave light yellow solids. They were recrystallized from ethanol to give light yellow solid in good yields.

7-fluoro-6-methoxy-1-(4-methoxyphenyl)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (7a): Color: light yellow solid. Yield: 56%. M.P: 142-144°C. IR (KBr,v,Cm⁻¹): 3397 (Carboxylic -OH), 1715 (carboxyl C=O), 1703 (tetralone C=O), 1599 (Aromatic C=C); 1H NMR (CDCl₃-400MHz) δ ppm: 2.28 (dd, 2H, -CH₂), 3.56 (q, 1H, -CH), 3.79 (s, 6H, -OCH₃), 4.8 (d, 1H, -CH), 7.2-7.34 (m, 6H, Ar-H); 13C NMR (CDCl₃-100 MHZ) δ ppm: 24.4, 35.5, 37.8, 44.3, 47.4, 96.9, 115.4, 117.8, 129.4, 130.9, 135.4, 143.3, 158.0, 158.4, 159.3, 179.2, MS (ESI, m/z): 329.11. Found: C, 65.98; H, 4.93 %.

7-fluoro-6-methoxy-4-oxo-1-phenyl-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (7b): Color: slight yellow solid. Yield: 54%. M.P: 127-130°C. IR (KBr,v,Cm⁻¹): 3397 (Carboxylic -OH), 1718 (carboxyl C=O), 1710 (tetralone C=O), 1598 (Aromatic C=C); 1H NMR (CDCl₃-400MHZ) δ ppm: 2.25 (dd, 2H, -CH₂), 3.42 (q, 1H, -CH), 3.77 (s, 3H, -OCH₃), 4.67 (d, 1H, -CH), 6.8-7.1 (m, 7H, Ar-H); 13C NMR (CDCl₃-100 MHZ) δ ppm: 38.5, 44.4, 47.4, 55.9, 117.8, 126.7, 128.4, 129.4, 131.7, 133.3, 144.3, 147.8, 157.4, 179.4, 197.4. MS (ESI, m/z): 315.19. Found: C, 68.78; H, 4.81 %.

7-fluoro-6-methoxy-4-oxo-1-(p-tolyl)-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (7c): Color: light yellow solid. Yield: 57%. M.P: 145-147°C. IR (KBr,v,Cm⁻¹): 3400 (Carboxylic -OH), 1718 (carboxyl C=O), 1705 (tetralone C=O), 1594 (Aromatic C=C); 1H NMR (CDCl₃-400MHZ) δ ppm: 2.22 (s, 3H, -CH₃), 2.33 (dd, 2H, -CH₂), 3.67 (q, 1H, -CH), 3.74 (s, 3H, -OCH₃), 4.55 (d, 1H, -CH), 7.08-7.44 (m, 6H, Ar-H); 13C NMR (CDCl₃-100 MHZ) δ ppm: 25.2, 38.2, 43.8, 44.7, 56.7, 127.2, 128.7, 130.7, 133.4, 137.5, 141.2, 148.2, 158.5, 179.5, 196.5. MS (ESI, m/z): 329.11. Found: C, 69.45; H, 5.18 %.

1-(3,4-dimethoxyphenyl)-7-fluoro-6-methoxy-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (7d): Color: slight yellow solid. Yield: 61%. M.P: 117-119°C. IR (KBr,v,Cm⁻¹): 3390 (Carboxylic -OH), 1718 (carboxyl C=O), 1705 (tetralone C=O); 1H NMR (CDCl₃-400MHZ) δ ppm: 2.36 (dd, 2H, -CH₂), 3.68 (q, 1H, -CH), 3.77 (s, 9H, -OCH₃), 4.7 (d, 1H, -CH), 6.9-7.44 (m, 5H, Ar-H); 13C NMR (CDCl₃-100 MHZ) δ ppm: 38.5, 44.2, 46.6, 56.7, 114.3, 116.7, 117.2, 122.7, 127.4, 131.7, 137.5, 137.4, 147.9, 148.3, 151.2, 158.2, 179.1, 197.7. MS (ESI, m/z): 375.19. Found: C, 64.13; H, 5.07 %.

2.3. Antimitotic assay

The novel tetralone acid analogues of podophyllotoxin (7a-e) were screened for antimitotic activity by onion root tip method and ID₅₀ value 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 ppm). 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 [13]. 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 following method of Fissceja [14]. The mitotic index was calculated by examination of minimum of zone cells. Three replicates were made for each calculation. The slides were observed under microscope and photographed.

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 (ID₅₀) was extrapolated from the graph of the concentration verses percentage inhibition. ID₅₀ values for novel tetralone acid analogues of podophyllotoxin for antimitotic activity were calculated individually following hakala [15] method.

3. RESULTS AND DISCUSSION
3.1. Chemistry

The compounds (7a-d) were synthesised by Gensler’s method with slight changes in reagent and experimental procedure (Scheme 1). Compounds (2a-d) were obtained by Freidel craft acylation of commercially available materials such as substituted benzyol chloride (1a-d) in dichloromethane and 2-flouro anisole in presence of anhyd. aluminum chloride \[16\]. Notably, this reaction occurred to give high yields of products. The compounds (2a-d) were converted to (3a-d) by stobbe condensation reaction using sodium hydride as strong base in benzene with diethyl succinate and ethanol \[17\]. The stobbe condensation reaction occurred smoothly at room temperature to obtain mixture of cis and trans isomers with good yields. The saponification of compounds (3a-d) was carried out with sodium hydroxide in water and methanol to form compounds (4a-d) in cis and trans isomeric form. The compounds (5a-d) were prepared by the catalytic hydrogenation of compounds (4a-d) in the presence of palladium over carbon in methanol under 3-4 kg hydrogen pressure at 25-30°C \[18, 19\]. The hydrogenated compounds (5a-d) were converted into benzhydryl succinic anhydrides (6a-e) using acetyl chloride under reflux condition. Tetralone acids (7a-e) were prepared by the intramolecular Friedel-Craft’s acylation reaction of benzhydryl succinic anhydrides in the presence of Lewis acids anhyd. aluminium chloride in dichloromethane \[20, 21\]. The products were characterized by IR, \(^{1}H\) NMR, \(^{13}C\) NMR, mass spectral and elemental analysis data.

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 C causes a certain changes in activity. The compound 7d has dimethoxy moiety on ring C, which is accounted for the enhanced antimitotic activity than when compared to control solution. On the other hand, the remaining compound 7b and 7c has hydrogen and methyl moiety showed less activity and compound 7a has methoxy exhibited moderate activity compared to control. From the obtained results, it is clear that the major role for antimitotic activity is played by substituents on ring C moiety. It is evident that novel tetralone acid analogues of podophyllotoxin were showed good antimitotic activity.

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**Scheme - 1: Protocol for the synthesis of new substituted tetralone acids.**
Table - 1: Antimitotic activity of the compounds (7a-d) by onion root tip method.

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4. CONCLUSION

In conclusion, we have synthesized new podophyllotoxin analogues (7a-d) in six step process in good yields using less expensive and readily available chemicals. Substituted benzophenone can be easily converted into itaconic half esters using sodium hydride as strong base via stobbe condensation reaction. The double bond of the α,β-unsaturated acids can be easily reduced to benhydrol succinic acids via hydrogenation in autoclave. The structures of synthesized compounds were confirmed and characterized by analytical and spectral data. All the synthesized analogues of podophyllotoxin (7a-d) were identified as antimitotic. For antimitotic activity, among the synthesized analogues, compound 7d showed more inhibition and exhibited high antimitotic activity. Compound 7b and 7c showed less activity where compound 7a exhibited moderate activity.

Acknowledgement

The authors are thankful to Institute of Excellence (IOE), University of Mysore, Mysore for providing spectral data.

5. REFERENCES

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