SYNTHESIS OF NEW PODOPHYLLOTOXIN ANALOGUES BEARING BARBITONE AND THIOBARBITONE MOIETY AS ANTIMITOTIC AGENTS

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Abstract: The new podophyllotoxin analogues bearing barbitone and thiobarbitone moiety were prepared. They were prepared in good yields by the reaction of tetralone with substituted benzaldehydes in 50% ethanolic sodium hydroxide followed by heating with barbituric acid and thiobarbituric acid in acetic acid and ethanol mixture. They were screened for antimitotic activity.

***Keywords*:** Podophyllotoxin, chalcone, tetralone, antimitotic activity, barbitone and thiobarbitone.

INTRODUCTION

Podophyllotoxin is a naturally occurring cyclolignan exhibits antineoplastic and antiviral properties [1]. Its antimitotic activity proved to be of the greatest interest to researchers [2]. Because of its toxic side effects, extensive structural modifications were performed since the 1950s. Podophyllotoxin derivatives possess antitumor activity, such as etoposide and teniposide have been widely used as anticancer drugs for clinical chemotherapy [3]. However, their low water solubility, acquired drug-resistance and severe gastrointestinal disturbances have promoted the search for new derivatives of podophyllotoxin [4]. The structural modifications and mechanism of action of podophyllotoxin have been studied over the years and the C4 position is considered potentially the most modifiable position. Diverse analogs like GL-331, NPF, TOP-53, NK-611, which are presently under clinical trial have been synthesized [5–7]. Podophyllotoxin can be semisynthesized as chemotherapeutic agent against different types of cancers [8] such as cervical carcinoma [9] osteosarcoma, nasopharyngeal carcinoma [10], colon cancer [11], breast cancer, prostate cancer [12], small cell lung cancer and testicular carcinoma [13, 14].

Chalcones are abundant in edible plants and are synthetic compounds belonging to the family of flavonoids which are prepared by Claisen–Schmidt condensation reaction of appropriate aldehydes with methyl ketones in the presence of base or acid followed by dehydration. Chalcones are an important group of natural products that consist of two aromatic rings joined by an α,β-unsaturated carbonyl system. The α,β-unsaturated carbonyl system enables chalcones and their heteroanalogs to undergo conjugated addition reactions in the presence of Lewis acid and basic catalysts [15, 16]. They possess a wide range of biological activities and industrial applications. They exhibit biological activities, such as antimicrobial, anticancer, antiprotozoal, antiulcer, anti-inflammatory and other activities [17-20].

The condensed thiobarbiturates exhibits diverse pharmacological activities such as antimicrobial, selective cell adhesion inhibitors and DNA cleavage activities [21]. Additionally, recent literature survey has indicated that barbituric acid derivatives may also act as immune modulators [22, 23]. Barbiturate and thiobarbiturate derivatives attracted considerable attention owing to their various biological effects such as inhibiting collagenase-3 (MMP-3) [24], recombinant cytochrome P450 enzymes [25], methionine aminopeptidase-1 (MetAP-1) [26], anti-inflammatory, analgesic [27], CYP19 inhibitory activity, molecular docking [28], cytotoxicity properties [29] and broad spectrum of pharmacological properties including hypnotic [30] and sedative [31].
Because of biological importance of podophyllotoxin and chalcone containing barbitone and thiobarbitone, the synthesis and evaluation of biological activity of podophyllotoxin analogues has been the objective of many research groups and continue being in expansion.

EXPERIMENTAL

Materials and methods

Melting point of the synthesized compounds **(5a-d)** and **(6a-d)** was determined by open capillary method and is uncorrected. The IR spectra were recorded on a FT-IR instrument in KBr disc. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Agilent 400MR DD2 spectrometer using TMS as an internal standard and dimethyl sulfoxide (DMSO)-*d6* as solvent. Chemical shifts and coupling constants are given in ppm (δ values) and Hz (J values). Elemental analyses were performed with a Perkin-Elmer 2400 instrument and were consistent with theoretical values within 0.4%. The mass spectra were recorded by Waters, USA on Synapt G2 HDMS/ACQUITY UPLC instrument. The purity of the compounds was checked by thin layer chromatography 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 stationery phase and benzene as the mobile phase. All the solvents and reagents were analytically pure, and further purification is not necessary.

Synthesis

General procedure for synthesis of chalcones (3a-d)

4-(3,4-Dichlorophenyl)-1-tetralone **(1)** (0.3 g, 1 mmol) was added a solution of 50% ethanolic sodium hydroxide (5 mL), then the aromatic aldehydes **(2a-d)** (1 mmol) was added gradually at 0 °C. The reaction mixture was then allowed to room temperature and stirred for 1 h. The precipitate is formed and then kept overnight in an ice bath. The precipitated products were filtered and washed with ice cold water and recrystallized from ethanol.

2-benzylidene-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2H)-one ***(3a)***

Color: Light yellow solid, m.p: 106-108 °C, Yield 81%, IR (KBr) υ_{max}(cm^{-1}): 1664 (C=O), 1551 (C=C), 1586 (HC=CH); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 7.02-7.65 (m, 12H, Ar-H), 6.93 (s, 1H, CH), 3.05 (t, 1H, *J*=3 Hz, CH), 2.43 (d, 2H, *J*=3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 187.9, 142.5, 141.6, 138.6, 137.3, 135.2, 135.0, 133.0, 132.5, 130.9, 130.7, 129.4, 128.7, 128.6, 128.5, 127.9, 127.7, 126.7, 41.9, 32.7; MS (*ESI*) m/z: 379.21 (M^+); Anal. calcd. for $C_{23}H_{16}Cl_2O$: C, 72.83; H, 4.25; Found: C, 72.82; H, 4.22%.

4-(3,4-dichlorophenyl)-2-(4-methylbenzylidene)-3,4-dihydronaphthalen-1(2H)-one ***(3b)***

Color: Yellow solid, m.p: 112-114 °C, Yield 78%, IR (KBr) υ_{max}(cm^{-1}): 1661 (C=O), 1556 (C=C), 1589 (HC=CH); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 7.05-7.60 (m, 11H, Ar-H), 6.86 (s, 1H, CH), 4.06 (t, 1H, CH), 2.54 (t, 3H, *J*=3 Hz, CH_2)2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 187.9, 142.5, 141.6, 138.6, 137.6, 137.3, 135.0, 133.0, 132.5, 130.9, 130.7, 129.4, 128.9, 128.7, 128.5, 127.9, 127.7, 126.7, 41.9, 32.7, 21.3; MS (*ESI*) m/z: 393.10 (M^+); Anal. calcd. for $C_{24}H_{18}Cl_2O$: C, 72.83; H, 4.25; Found: C, 73.79; H, 4.27%.

4-(3,4-dichlorophenyl)-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one ***(3c)***

Color: Yellowish white solid, m.p: 108-110 °C, Yield 86%, IR (KBr) υ_{max}(cm^{-1}): 1665 (C=O), 1562 (C=C), 1581 (HC=CH); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 7.10-7.54 (m, 11H, Ar-H), 7.01 (s, 1H, CH), 4.11 (t, 1H, CH), 3.79 (s, 3H, OCH_3), 2.23 (t, 2H, *J*=3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 187.9, 159.8, 142.5, 141.6, 138.6, 137.6, 137.3, 135.0, 133.0, 132.5, 130.9, 130.7, 130.2, 129.4, 128.7, 127.9, 127.7, 127.5, 114.2, 55.8, 41.9, 32.7; MS (*ESI*) m/z: 409.24 (M^+); Anal. calcd. for $C_{24}H_{18}Cl_2O_2$: C, 70.43; H, 4.43; Found: C, 70.46; H, 4.42%.

4-(3,4-dichlorophenyl)-2-(4-(methylthio)benzylidene)-3,4-dihydronaphthalen-1(2H)-one ***(3d)***

Color: Greenish brown, m.p: 122-124 °C, Yield 72%, IR (KBr) υ_{max}(cm^{-1}): 1670 (C=O), 1566 (C=C), 1592 (HC=CH); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 7.06-7.62 (m, 11H, Ar-H), 6.94 (s, 1H, CH), 4.03 (t, 1H, CH), 2.64 (s, 3H, SCH_3), 2.23 (d, 2H, *J*=3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 187.9, 142.5, 141.6, 138.6, 137.6, 137.3, 135.0, 133.0, 132.5, 130.9, 130.7, 131.6, 129.4, 128.9, 128.7, 127.9, 127.7, 126.7, 114.2, 41.9, 32.7, 14.8; MS (*ESI*) m/z: 425.42 (M^+); Anal. calcd. for $C_{24}H_{18}Cl_2OS$: C, 67.77; H, 4.27; Found: C, 67.79; H, 4.24%.

General procedure for the synthesis of new podophyllotoxin analogues bearing barbitone (5a-d) and thiobarbitone moiety (6a-d)

To the ethanolic solution of 4-(3,4-dichlorophenyl)-1-tetralone chalcones **(3a-d)** (1 mmol) at room temperature, barbituric acid (1.2 mmol) or thiobarbituric acid **(4)** (1.2 mmol) was added slowly over a 20 min. and then catalytic amount of AcOH was added. The reaction mixture was refluxed for 7 h. After completion of reaction, the reaction mixture was cooled to room temperature and quenched with 2M HCl in an ice bath and then neutralized with $NaHCO_3$ solution. The precipitated solid was collected by filtration and recrystallized from ethanol.

5-(2-benzylidene-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2H)-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ***(5a)***

Color: Yellowish white solid, m.p: 160-162 °C, Yield 64%, IR (KBr) υ_{max}(cm^{-1}): 3315 (NH), 1683, 1650, 1659 (C=O); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.74 (s, 2H, NH), 7.31-7.60 (m, 12H, Ar-H), 3.18 (t, 1H, *J*=2.2 Hz, CH), 2.11 (dd, 2H, *J*=2.3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 164.9, 161.0, 150.4, 142.5, 139.9, 138.1, 135.2, 133.8, 132.5, 130.9, 130.7, 129.4, 128.6, 128.5, 128.4, 128.1, 127.9, 127.7, 126.9, 126.1, 125.7, 120.3, 42.5, 39.4; MS (*ESI*) m/z: 488.93 (M^+); Anal. calcd. for $C_{27}H_{18}Cl_2N_2O_3$: C, 66.27; H, 3.71; N, 5.72; Found: C, 66.24; H, 3.72; N, 5.75%.

5-(4-(3,4-dichlorophenyl)-2-(4-methylbenzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ***(5b)***

Color: Brownish yellow , m.p: 168-170 °C, Yield 69%, IR (KBr) υ_{max}(cm^{-1}): 3311 (NH), 1672, 1654, 1663 (C=O); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.84 (s, 2H, NH), 7.34-7.67 (m, 11H, Ar-H), 3.15 (t, 1H, *J*=2.4 Hz, CH), 2.01 (dd, 2H, *J*=2.3 Hz, CH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 164.9, 161.0, 150.4, 142.5, 139.9, 138.1, 137.6, 133.8, 132.5, 132.2, 130.9, 130.7, 129.4, 128.9, 128.5, 128.4, 128.1, 127.7, 126.9, 126.1, 125.7, 120.3, 42.5, 39.4, 21.3; MS (*ESI*) m/z: 502.12 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_3$: C, 66.81; H, 4.00; N, 5.57; Found: C, 66.83; H, 4.01; N, 5.54%.

5-(4-(3,4-dichlorophenyl)-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ***(5c)***

Color: Greenish yellow solid, m.p: 155-157 °C, Yield 72%, IR (KBr) υ_{max}(cm^{-1}): 3321 (NH), 1676, 1651, 1665 (C=O); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.68 (s, 2H, NH), 7.30-7.72 (m, 11H, Ar-H), 3.18 (t, 1H, *J*=2.9 Hz, CH), 2.15 (dd, 2H, *J*=2.3 Hz, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 164.9, 161.0, 159.8, 150.4, 142.5, 139.9, 138.1, 133.8, 132.5, 130.9, 130.7, 130.2, 128.4, 128.1, 127.7, 127.5, 126.9, 125.7, 126.1, 120.3, 129.4, 114.2, 55.8, 42.5, 39.4; MS (*ESI*) m/z: 519.01 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_4$: C, 64.75; H, 3.88; N, 5.39; Found: C, 64.76; H, 3.85; N, 5.42%.

5-(4-(3,4-dichlorophenyl)-2-(4-(methylthio)benzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ***(5d)***
Color: Brown solid, m.p: 150-152 ºC, Yield 62%, IR (KBr) υ_{max}(cm^{-1}): 3328 (NH), 1673, 1656, 1660 (C=O); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.62 (s, 2H, NH), 7.35-7.75 (m, 11H, Ar-H), 3.10 (t, 1H, *J*=2.1 Hz, CH), 2.18 (dd, 2H, *J*=2.3 Hz, CH_2), 2.54 (s, 3H, SCH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 164.9, 161.0, 150.4, 142.5, 139.9, 138.6, 138.1, 133.8, 132.5, 131.6, 130.9, 130.7, 129.4, 128.9, 128.4, 128.1, 127.7, 126.7, 126.9, 126.1, 125.7, 120.3, 42.5, 39.4, 14.8; MS (*ESI*) m/z: 535.14 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_3S$: C, 62.81; H, 3.76; N, 5.23; Found: C, 62.80; H, 3.75; N, 5.20%.

5-(2-benzylidene-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2H)-ylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione ***(6a)***
Color: Greenish yellow solid, m.p: 164-166 ºC, Yield 61%, IR (KBr) υ_{max}(cm^{-1}): 3315 (NH), 1679, 1668 (C=O), 1348 (C=S); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.74 (s, 2H, NH), 7.34-7.62 (m, 12H, Ar-H), 3.14 (t, 1H, *J*=2.3 Hz, CH), 2.04 (dd, 2H, *J*=2.3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 175.2, 166.9, 161.0, 142.5, 139.9, 138.1, 135.2, 133.8, 132.5, 130.9, 130.7, 129.4, 128.6, 128.5, 128.4, 128.1, 127.9, 127.7, 126.9, 126.1, 125.7, 120.3, 42.5, 39.4; MS (*ESI*) m/z: 504.02 (M^+); Anal. calcd. for $C_{27}H_{18}Cl_2N_2O_2S$: C, 64.16; H, 3.59; N, 5.54; Found: C, 64.13; H, 3.55; N, 5.50%.

5-(4-(3,4-dichlorophenyl)-2-(4-methylbenzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione ***(6b)***
Color: Brownish black, m.p: 159-161 ºC, Yield 58%, IR (KBr) υ_{max}(cm^{-1}): 3322 (NH), 1686, 1660 (C=O), 1354 (C=S); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.71 (s, 2H, NH), 7.21-7.75 (m, 11H, Ar-H), 3.12 (t, 1H, *J*=2.3 Hz, CH), 2.18 (dd, 2H, *J*=2.3 Hz, CH_2), 2.23 (s, 3H, CH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 175.2, 166.9, 161.0, 142.5, 139.9, 138.1, 137.6, 133.8, 132.5, 132.2, 130.9, 130.7, 129.4, 128.9, 128.5, 128.4, 128.1, 127.7, 126.9, 126.1, 125.7, 120.3, 42.5, 39.4, 21.3; MS (*ESI*) m/z: 518.11 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_2S$: C, 64.74; H, 3.88; N, 5.39; Found: C, 64.75; H, 3.86; N, 5.35%.

5-(4-(3,4-dichlorophenyl)-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione ***(6c)***
Color: Yellow solid, m.p: 168-170 ºC, Yield 75%, IR (KBr) υ_{max}(cm^{-1}): 3326 (NH), 1693, 1663 (C=O), 1350 (C=S); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.68 (s, 2H, NH), 7.15-7.50 (m, 11H, Ar-H), 3.20 (t, 1H, *J*=2.2 Hz, CH), 2.19 (dd, 2H, *J*=2.3 Hz, CH_2), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 175.2, 166.9, 161.0, 159.8, 142.5, 139.9, 138.1, 133.8, 132.5, 130.9, 130.7, 130.2, 129.4, 128.4, 128.1, 127.7, 127.5, 125.7, 126.9, 126.1, 120.3, 114.2, 55.8, 42.5, 39.4; MS (*ESI*) m/z: 536.21 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_3S$: C, 62.81; H, 3.76; N, 5.23; Found: C, 62.80; H, 3.75; N, 5.21%.

5-(4-(3,4-dichlorophenyl)-2-(4-(methylthio)benzylidene)-3,4-dihydronaphthalen-1(2H)-ylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione ***(6d)***
Color: Greenish black solid, m.p: 154-156 ºC, Yield 60%, IR (KBr) υ_{max}(cm^{-1}): 3316 (NH), 1678, 1661 (C=O), 1344 (C=S); ^{1}H NMR (DMSO-*d6* 400 MHz) δ ppm: 8.60 (s, 2H, NH), 7.26-7.70 (m, 11H, Ar-H), 3.84 (t, 1H, *J*=2.5 Hz, CH), 2.44 (s, 3H, SCH_3), 2.08 (dd, 2H, *J*=2.3 Hz, CH_2); ^{13}C NMR (DMSO-*d6* 100 MHz) δ ppm: 175.2, 166.9, 161.0, 142.5, 139.9, 138.6, 138.1, 131.6, 133.8, 132.5, 130.9, 130.7, 129.4, 128.9, 128.4, 128.1, 127.7, 126.9, 126.7, 126.1, 125.7, 120.3, 42.5, 39.4, 14.8; MS (*ESI*) m/z: 551.26 (M^+); Anal. calcd. for $C_{28}H_{20}Cl_2N_2O_2S_2$: C, 60.98; H, 3.66; N, 5.08; Found: C, 61.00; H, 3.64; N, 5.04%.

Antimitotic activity
The antimitotic activity of new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d)** was evaluated using onion root tip method, and the % inhibition was determined. 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 (0.1 mg/mL). To study the effect of new podophyllotoxin analogues bearing barbitone and thiobarbitone moiety on somatic cells, onion base was immersed to an extent of about half a centimeter in a sample tube and control solution tube (7X3), after removing the old roots from it, and immersion is continued for 12 h intervals respectively for germination. After this, 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 persevered 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). The glasses were warmed and kept for 1 h. The roots were taken on a clean glass slide and squashed using 45 % acetic acid following the method of Levan [32]. 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 method [33]. 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.

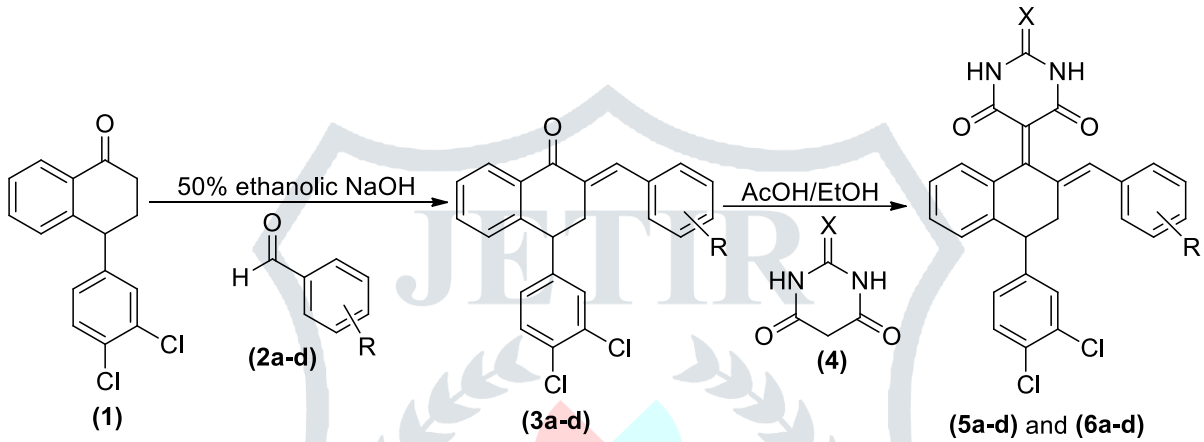
$$MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} X\ 100$$

The percentage of the number of dividing cells compared to the control and the percent inhibition of mitosis by antimitotic agent at concentration (0.1 mg/mL) against a control was calculated [34].

RESULTS AND DISCUSSION
Chemistry
The synthesis of new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d)** has been carried out by chalcone route (Scheme 1). As shown in **scheme 1**, commercially available 4-(3,4-Dichlorophenyl)-1-tetralone **(1)** was treated with aromatic aldehydes **(2a-d)** in the presence of 50% ethanolic NaOH solution to give 4-(3,4-Dichlorophenyl)-1-tetralone chalcones **(3a-d)** [35]. The structures of the chalcones were confirmed by IR and ^{1}H NMR spectral studies. IR spectra of

compounds **(3a–d)** showed the C=C stretching frequency in the range 1581-1592 cm^{-1} and ^1H NMR showed the absence of aldehyde proton at 9.81 ppm.

The key intermediates, 4-(3,4-Dichlorophenyl)-1-tetralone chalcones **(3a-d)** was then undergo by Knoevenagel condensation with barbituric acid and thiobarbituric acid to afford target compounds **(5a-d)** and **(6a-d)** [36]. Initially, 4-(3,4-Dichlorophenyl)-1-tetralone chalcones undergo protonation by acetic acid. The protonated form of the methanone then facilitates the addition reaction towards a nucleophile. The acetate ion which was formed in the former step can accept a proton from the methylene unit of barbituric acid and thiobarbituric acid and generate a carbanion. The electron-rich carbanion attacks on the electron deficient carbonyl carbon of 4-(3,4-Dichlorophenyl)-1-tetralone chalcones to form an adduct which on dehydration furnished the target compounds. The IR spectrum of the synthesized compounds showed sharp absorption bands at 1659-1668 cm^{-1}, 1328-1354 cm^{-1} and 3311-3328 cm^{-1} corresponding to (C=O), (C=S) and (NH) stretching vibration respectively. The ^1H NMR spectrum of target compounds displayed a doublet at δ 6.10-6.84 ppm due to one vinyl protons, the multiplet between δ 7.15-7.75 ppm correspond to aromatic protons and a singlet at δ 8.60-8.84 ppm is due to two NH protons. Further, ^{13}CNMR spectrum of compound 5a confirmed the proposed structure by appearance of signal at δ 150.4 ppm due to the –NHCONH- carbon of barbituric acid ring and another signal at δ 175.2 ppm correspond to –NHCSNH- carbon of thiobarbituric acid ring. Another signal at δ 132.5 and δ 130.9 ppm attributed to two C-Cl carbon and rest of carbon atoms displayed the signals at respective δ values pertaining to the structure.



Scheme 1. Protocol for synthesis of new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d)**
5a: X=O: R=H; **5b**: X=O: R=CH_3; **5c**: X=O: R=OCH_3; **5d**: X=O: R=SCH_3
6a: X=S: R=H; **6b**: X=S: R=CH_3; **6c**: X=S: R=OCH_3; **6d**: X=S: R=SCH_3

Antimitotic activity
Allium Cepa has been used to evaluate antimitotic activity of new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d)** by onion root tip method.

To evaluate the antimitotic activity of the synthesized new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d)**, all the eight compounds **(5a-d)** and **(6a-d)** were tested for their antimitotic activity compared with control. Onion roots in synthesized compounds of 0.1 mg/mL at 24 h exhibited changes in chromosomes and shape of the cells with elongated appearance. The comparative antimitotic study of the target compounds is in the form of their % inhibition values has been presented in Table 1. It is more attractive to speculate the observation that the result of the antimitotic activity of new podophyllotoxin analogues bearing barbitone or thiobarbitone moiety appeared to be related to barbitone or thiobarbitone unit and substituents on the phenyl ring.

The present study demonstrates that nature of the lactone ring is modified with barbitone and thiobarbitone moiety showed less antimitotic activity and higher activity by changing the substituents on phenyl ring of chalcone moiety exert a decisive influence on the antimitotic activity of compounds. The most promising compound **6d** contained a thiobarbitone moiety and electron donating thiomethyl group at *para* position of chalcone ring. In comparison to control better inhibition were obtained for compounds **6b** and **6c** having thiobarbitone moiety at the lactone ring and methyl and methoxy group on chalcone ring at *para* position respectively except **5a, 5b, 5c, 5d** and **6a** displayed less antimitotic activity compared to control. In general, all the above compounds exhibited better antimitotic property than those reported in our earlier papers [35, 37-39].

Table 1. % Inhibition of new podophyllotoxin analogues bearing barbitone **(5a-d)** and thiobarbitone moiety **(6a-d).**

Compounds	Entry X	Entry R	% Dividing cells	% Dividing cells compared to control	% Inhibition
Control	-	-	39.13	100	00
5a	O	H	13.89	35.49	64.51
5b	O	CH_3	13.01	34.24	66.76
5c	O	OCH_3	12.25	31.30	68.70
5d	O	SCH_3	12.04	30.76	69.24
6a	S	H	11.06	28.26	71.74
6b	S	CH_3	8.56	21.87	78.13
6c	S	OCH_3	8.45	21.59	78.41
6d	S	SCH_3	8.08	24.64	79.36

CONCLUSION

A series of new podophyllotoxin analogues bearing barbitone and thiobarbitone moiety were prepared using chalcone method and they have been evaluated for their antimitotic activity by onion root method. The antimitotic studies displayed that new podophyllotoxin analogue **6d** possessed potential antimitotic activity towards control with % inhibition values of 79.36 and **6b** and **6c** exhibited better % inhibition values of 78.13 and 78.41 respectively, while **5a, 5b, 5c, 5d** and **6a** showed less % inhibition values of 64.51, 66.76, 68.70, 69.24 and 71.74 respectively.

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