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Synthesis and evaluation of biological activity of nitrogen and oxygen containing heterocyclic analogues of podophyllotoxin

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ABSTRACT

A novel nitrogen containing analogues of podophyllotoxin 6a-d to 9a-d i.e., substituted 5-phenyl-2,4,5,7,8,9-hexahydroindeno[5,6-g]indozole were synthesized in high yields by the condensation of hydroxylmethylene tetralones and hydrazine hydrate, hydroxyl amine, urea, thiourea in absolute ethanol. The structures of the compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectral and elemental analysis data. The new compounds were evaluated for their antimitotic activity by onion root tip method and also antimicrobial activity against bacterial and fungal strains. The antimitotic activity was compared with control, compound 7b, 8d showed more potent inhibitions and compound 7a,7c, 8b, 8c, 9b,9c,9d exhibited moderate inhibitions. The antimicrobial activity of the synthesized compounds were compared with standards, compound 6b,8b,9b showed excellent activity and compound 7b showed moderate activity against bacterial and fungal strains. In the antimicrobial activity, among the synthesized analogues 6a-d to 9a-d compound 9b showed high activity against all the bacterial and fungal strains. Hence, compound 7b showed very good activity in antimitotic and 9b antimicrobial activity.

Keywords: Acetophenones, Chalcones, Cyclopropyl ketones, Tetralones, Antimitotic activity, Antimicrobial activity.

1. INTRODUCTION

Many methods have been reported in the literature for the synthesis of podophyllotoxin and its analogues. However, most of the reaction procedures suffer from several drawbacks such as long reaction times, harsh reaction conditions, non-commercially available materials and tedious work-up procedures. It was decided to synthesize novel nitrogen containing analogues of podophyllotoxin by chalcone method [1]. Podophyllotoxin 1 is a bioactive lignan which has been isolated from the roots of podophyllum peltatum. Although the therapeutic application of podophyllotoxin is limited to topical use due to its high toxicity, its semisynthetic derivatives etoposide 2 and teniposide 3 have been widely used as important anticancer drugs in clinical use, which has prompted extensive structural modification, particularly at the *C4* position of podophyllotoxin. The *C4* derivatives, GL-331 4 and TOP-53 5, have displayed unique antitumor activity and reached clinical trials figure 1 [2]. The clinical use of podophyllotoxin molecule is limited

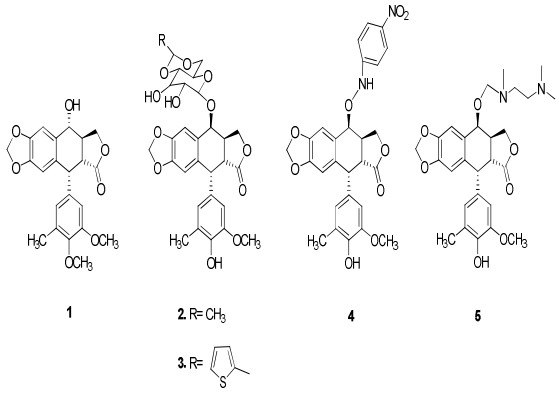


Figure - 1: Structure of Podophyllotoxin 1, Etoposide 2, Teniposide 3, GL-331 4 and TOP-53 5.

due to its very high toxic nature [3] which causes side effects like nausea, diaherria, damages to healthy tissues and vomiting [4]. Further, its cytotoxic nature can be reduced by doing some modifications to the structure. Extensive structural modifications for podophyllotoxin were

reported in the literature [5,6]. Since podophyllotoxin is having ability to inhibit the growth of cancerous cells, hence it is known as a very good anticancer agent [7].

In view of this, the novel nitrogen containing analogues of podophyllotoxin were synthesized and their biological activities were studied.

2. MATERIALS AND METHODS

All reagents and 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 a developing agent. Acme, India silica gel, 60–120 mesh is used for column chromatography. 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 trimethyl 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.

2.1. SYNTHESIS

2.1.1. Procedure for the synthesis of 1-(2, 3-Dihydro-1H-inden-5-yl)ethanone (1a)

5-Acetylindane was prepared in good yield by Friedel-Craft's acylation reaction of Indane with acetic anhydride in presence of fused zinc chloride. The reaction mixture was stirred for 12 hours at room temperature.

1-(2, 3-Dihydro-1H-inden-5-yl)ethanone

Color: Thick red oily liquid. Yield**:** 87.66%. IR (KBr, ν, cm^{-1}): 2988 (Ar-CH), 1650 (C=O), 1590 (C=C); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.81 (d, 1H, Ar-H) 7.31 (s, 1H, Ar-H), 2.89-2.85 (t, 4H) 2.56 (s, 3H –CH_3), 2.00 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 198.2, 150, 144.4, 135.6, 126.1, 125.9, 32.8, 26.7,25.3 MS (ESI) m/z: 160.09 (M^+). *Anal*. Calcd. for $C_{11}H_{12}O$: C, 82.46, H, 7.55. Found: C, 82.45, H, 7.53%

2.1.2. General procedure for the synthesis of chalcones (2a-d)

1-(2, 3-Dihydro-1H-inden-5-yl)ethanone (0.05 mol) (1a**)** and substituted benzaldehyde (6.68 ml, 0.05 mol) were stirred in water (40 ml) and ethanol (25 ml) mixture in the presence of sodium hydroxide (2.00 g, 0.06 mol) at 15-30 ^{0}C for 4 hrs. The reaction mixture was kept overnight in an ice bath. The precipitated products were filtered and recrystallized from ethanol.

1-(2,3-Dihydro-1H-inden-5-yl)-3-phenylprop-2-en-1-one (2a)

Color: yellow solid. Yield: 81.65 %. M.p.: 91-92 ^{0}C. IR (KBr, ν, cm^{-1}): 3368-2963 (Ar-CH), 1653 (C=O), 1591 (C=C); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.89 (d, 1H, *J* = 8.06 Hz, β-CH), 7.83-7.25 (m, 9H, Ar-H and α C-H), 2.99- 2.521(t, 4H), 2.16-2.09 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 198.2, 150.2, 144.7,135.6, 128.6, 127.5, 124.2, 32.8 25.3, MS (ESI) m/z: 248.15 (M^+). *Anal*. Calcd. for $C_{18}H_{16}O$: C, 87.06; H, 6.49. Found: C, 87.03; H, 6.45%.

1-(2,3-Dihydro-1H-inden-5-yl)-3-(p-tolyl)prop-2-en-1-one (2b)

Color: Light brown solid. Yield: 86.12 %. M.p.: 108-109^{0}C. IR (KBr, ν, cm^{-1}): 2960-2940 (Ar-CH), 1653 (C=O), 1588 (C=C); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.87(d, 1H, *J* = 7.5 Hz, β-CH), 7.82-7.18 (m, 8H, Ar-H and α C-H), 2.97-2.41(t, 4H) 2.32-2.10 (s, 3H CH_3), 2.07 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 190.5, 149.9, 144.8, 136.7, 132.3, 129.8, 128.4, 127.0, 121.3, 33.0, 25.4, 21.5, MS (ESI) m/z: 262.15 (M^+). *Anal*. Calcd. for $C_{19}H_{18}O$: C, 86.99; H, 6.92. Found: C, 86.97; H, 6.91%.

1-(2,3-Dihydro-1H-inden-5-yl)-3-(4-flurophenyl)prop-2-en-1-one (2c)

Color: Light yellow solid. Yield: 86.10 %. M.P.: 99-101 ^{0}C. IR (KBr, ν, cm^{-1}): 2981 (Ar-CH), 1656 (C=O), 1586 (C=C); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.87 (d, 1H, *J* = 7.5 Hz, β-CH), 7.82-7.10 (m, 8H, Ar-H and α C-H), 2.98 (t, 4H); 2.15-2.08 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 190.14, 162.6, 150.15, 144.9, 136.5, 130.3, 127.0, 124.4, 122.0, 115.9, 33.0, 25.38, MS (ESI) m/z: 266.11 (M^+). *Anal*. Calcd. for $C_{18}H_{15}FO$: C, 81.18; H, 5.68.Found: C, 81.15; H, 5.66%.

1-(2,3-Dihydro-1H-inden-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one (2d)

Color: Light yellow solid. Yield: 77.53 %. M.p.: 116-118 ^{0}C. IR (KBr, ν, cm^{-1}): 3130-2957 (Ar-CH), 1660 (C=O), 1601 (C=C); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.25 (d, 1H, *J* = 8.3 Hz, β-CH), 7.89-7.35 (m, 8H, Ar-H and α C-H), 2.99 (t, 4H), 2.16-2.05(m, 2H,); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 189.4, 150.8, 148.3, 145.1, 141.3, 135.9, 128.8, 126.2, 124.1, 32.9, 25.3, MS (ESI) m/z: 293.11 (M^+). *Anal*. Calcd. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15. Found: C, 73.72; H, 5.14%.

2.1.3. General procedure for the synthesis of cyclopropyl ketone (3a-d)

Sodium hydride (0.48 g of 0.02 mol) was added in portions to the stirred suspensions of trimethylsulfoxonium iodide (4.41 g of 0.02 mol) in dry benzene (20 ml) under nitrogen gas

atmosphere. The reaction mixture was stirred for 10 minutes at 25-30 ^{0}C (until the evolution of the H_2 gas ceased). Chalcones (0.02 mol) (2a-d) in dry benzene (15 ml) were added drop wise during 30 minutes to the above solution. The reaction mixture was stirred at 26-28 ^{0}C for 2 hours and raised the temperature to 50-60 ^{0}C for 1 hour. The completion of the reaction was confirmed by TLC. The reaction mixture was poured into water (20 ml). The precipitated gummy residue was extracted into chloroform. The combined organic layer was washed with water, dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. They were recrystallized from ethanol.

(2,3-dihydro-1H-inden-5-yl)(2-phenylcyclopropyl)methanone (3a)

Color: Dark brown solid. Yield: 72.78 %. IR (KBr, ν, cm^{-1}): 3058-2926 (Ar-CH), 1683 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.88-7.13 (m, 8H, Ar-H), 2.99-2.91 (t, 4H,), 2.89-2.10 (t, 2H) 1.29 (m, 2H) 0.85 (t, 2H, *J* = 7.3 Hz, cyclopropyl CH_2); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 1922, 147.3, 144.3, 141.5, 134.0, 127.9, 126.5, 126.0, 125.1, 32.8, 27.0, 25.3, 25.1, 14.6; MS (ESI) m/z: 262.14 (M^+). *Anal*. Calcd. for $C_{19}H_{18}O$: C, 86.99; H, 6.92. Found: C, 86.97, H, 6.91%.

(2,3-Dihydro-1H-inden-5-yl)(2-(p-tolyl) cyclopropyl) methanone (3b)

Color: Dark brown solid. Yield: 78.61 %. IR (KBr, ν, cm^{-1}): 2956-2853 (Ar-CH), 1683 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.87-7.18 (m, 7H, Ar-H), 2.97 (t, 4H), 2.36 (s, 3H, CH_3), 2.10-2.09 (m, 2H, cyclopropyl CH), 1.254 (m, 2H) 0.85 (t, 2H, *J* = 7.1 Hz, cyclopropyl CH_2); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 190.4, 149.8, 144.2, 136.7, 132.2, 129.6, 128.4, 126.9, 125.8, 124.4, 32.5, 29.7, 25.3, 21.4, 14.6 MS (ESI) m/z: 276.15 (M^+). *Anal*. Calcd. for $C_{20}H_{20}O$: C, 86.92; H, 7.29. Found: C, 86.90; H, 7.27%.

(2,3-Dihydro-1H-inden-5-yl)(2-(4-fluorophenyl) cyclopropyl) methanone (3c)

Color: Dark brown solid. Yield: 78.02 %. IR (KBr, ν, cm^{-1}): 2923-2853 (Ar-CH), 1682 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.92-7.20 (m, 7H, Ar-H), 2.91 (t, 4H) 2.10-2.03 (m, 2H, cyclopropyl CH), 1.66 (m, 2H) 0.83 (t, 2H, *J* = 7.5 Hz, cyclopropyl CH_2); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 191.4, 159.1, 147.4, 144.1, 137.2, 134.0, 128.1, 126.0, 125.5, 114.9, 32.8, 27.0, 25.3, 14.6; MS (ESI) m/z: 280.13 (M^+). *Anal*. Calcd. for $C_{19}H_{17}FO$: C, 81.40; H, 11.0,. Found: C, 81.39; H, 10.99%.

(2,3-Dihydro-1H-inden-5-yl)(2-(4-nitrophenyl)cyclopropyl)methanone (3d)

Color: Dark brown solid. Yield: 70.28 %. IR (KBr, ν, cm^{-1}): 2922-2853 (Ar-CH), 1683 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.28-7.26 (m, 7H, Ar-H), 2.99-2.22 (t, 4H), 2.18-2.11 (m, 2H, cyclopropyl CH), 1.29-0.89 (m, 2H) 0.83 (t, 2H, *J* = 7.2 Hz, cyclopropyl CH_2); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 189.4, 150.7, 148.4, 145.1, 141.2, 136.0, 126.1, 124.5, 124.1, 33.0, 29.6, 25.3, 25.1, 14.7 MS (ESI) m/z: 307.12 (M^+). *Anal*. Calcd. for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.58. Found: C, 74.23; H, 5.57%.

2.1.4. General procedure for the synthesis of tetralones (4a-d)

Cyclopropyl ketones (0.01 mol) *(3a-d)* was dissolved in dry dichloromethane (50 ml). Acetic anhydride (0.94 ml, 0.01 mol) and anhydrous stannic chloride (1.17 ml, 0.01 mol) were added under nitrogen gas atmosphere. The resultant reaction mixture was stirred at 25-28 ^{0}C for 3 hrs. The completion of reaction was known by TLC. The reaction mixture was poured into 5% NaOH solution (20 ml), the product was extracted into dichloromethane. The organic layer was washed with 5% HCl followed by water, dried over anhyd. Na_2SO_4 and concentrated under vacuum using a rotary evaporator to give brown residue. The product was purified by column chromatography using silica gel (60-120 mesh) as adsorbent and benzene as eluent. The benzene solution was concentrated to a small volume (20 ml) and hexane (100 ml) was added drop wise to give products in good yields. They were recrystallized from ethanol.

8-Phenyl-2, 3, 7, 8-tetrahydro-1H-cyclopenta[b]naphthalene-5(6H)-one (4a)

Color: Dark brown gummy solid. Yield: 67.34 %. IR (KBr, ν, cm^{-1}): 2923-2853 (Ar-CH), 1683 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.86-7.03 (m, 7H, Ar-H), 4.71 (t, 1H, *J* = 6.3 Hz, CH), 2.86-2.77 (t, 4H), 2.70-2.00 (tt, 4H, *J* = 6.1 Hz, *J* = 6.8 Hz, CH_2), 1.69-1.16 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 197.8, 149.1, 145.5, 144.6, 136.9, 131.3, 129.0, 128.1, 127.3, 125.2, 45.2, 37.3, 32.7, 30.6, 25.1; MS (ESI) m/z: : 262.14 (M^+). *Anal*. Calcd. for $C_{19}H_{18}O$: C, 86.99; H, 6.92. Found: C, 86.97; H, 6.90%.

8-(p-Tolyl)-2, 3, 7, 8-tetrahydro-1H-cyclopenta[b]naphthalene-5(6H)-one (4b)

Color: Dark brown gummy solid. Yield: 66.50 %. IR (KBr, ν, cm^{-1}): 2923-2853 (Ar-CH), 1679 (C=O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 7.98-6.84 (m, 6H, Ar-H), 4.24 (t, 1H, *J* = 6.4 Hz, CH), 2.89-2.73 (t, 4H), 2.61-2.25 (tt, 4H, *J* = 6.0 Hz, *J* = 6.2 Hz, CH_2),2.20 (s, 3H, -CH_3) 1.99-1.27 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 197.8, 149.4, 145.5, 142.0, 137.6, 131.2, 129.1, 129.3, 128.0, 127.1, , 37.3, 32.7, 30.7, 25.1, 21.2 MS (ESI) m/z: :

276.15 (M^+). *Anal*. Calcd. for $C_{20}H_{20}O$: C, 86.92; H, 7.29. Found: C, 86.90; H, 7.28%.

8-(4-Fluorophenyl)-2, 3, 7, 8-tetrahydro-1H-cyclopenta[b]naphthalene-5(6H)-one (4c)

Color: Dark brown gummy solid. Yield: 70.12 %. IR (KBr, ν, cm^{-1}): 2923-2853 (Ar-CH), 1680 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 7.24-6.90 (m, 6H, Ar-H), 3.71-3.68 (t, 1H, *J* = 6.5 Hz, CH), 2.88-2.81 (t, 4H), 2.33-2.10 (tt, 4H, *J* = 6.4 Hz, *J* = 6.3 Hz, CH_2), 2,01 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 197.8, 160.1, 149.5, 145.3, 140.1, 137.5, 131.1, 129.3, 129.0, 115.7, 45.2, 37.3, 32.7, 31.0, 25.1 MS (ESI) m/z: : 280.13 (M^+). *Anal*. Calcd. for $C_{19}H_{17}FO$: C, 81.40; H, 6.11. Found: C, 81.38; H, 6.09%.

8-(4-Nitrophenyl)-2, 3, 7, 8-tetrahydro-1H-cyclopenta[b]naphthalene-5(6H)-one (4d)

Color: Dark brown gummy solid. Yield: 60.16 %. IR (KBr, ν, cm^{-1}): 2923-2853 (Ar-CH), 1681 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 8.17-7.24 (m, 6H, Ar-H), 3.29 (t, 1H, *J* = 6.3 Hz, CH), 2.86 (t, 4H), 2.66-2.19 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.93 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 197.8, 151.1, 149.5, 145.5, 145.3, 137.5, 131.2, 129.1, 124.2, 121.0, 37.3, 32.7, 30.0, 25.1 ; MS (ESI) m/z: : 307.12 (M^+). *Anal*. Calcd. for $C_{19}H_{17}NO3$: C, 74.25; H, 5.58. Found: C, 74.23; H, 5.57%.

2.1.5. General procedure for the synthesis of substituted hydroxyl methylene tetralones 5a-d

Sodium hydride (1.2 g, 0.05 mol) was added to a mixture of absolute ethanol (10 ml) and dry benzene (150 ml) and stirred for 1 hr. Ethyle formate (10 ml) was added dropwise to the above reaction mixture and stirred for another 1 hr, followed by dropwise addition of tetralones (0.05 mol) 4a-d, in dry benzene (100 ml) over a period of 1 hr. After stirring the red coloured mixture at room temperature for 2 hrs, it was poured into 2N H_2SO_4 (100 ml) in ice. The separated organic layer was washed with water (3x50 ml) and extracted into 1 % sodium hydroxide solution (3x50 ml). The sodium hydroxide extract was acidified with 2N H_2SO_4 gave products in good yields. They were recrystallized from ethanol.

6-(hydroxymethylene)-8-phenyl-2,3,7,8-tetrahydro-1H-cyclopenta[b]naphthalene-5(6H)-one: (5a)

Color: Dark brown solid. Yield: 70.75 %. M.p.: 146-148 ^0C. IR (KBr, ν, cm^{-1}): 3391 (O-H), 2953-2853 (Ar-CH), 1713 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 14.20 (bs, 1H, OH vinyl), 7.71-7.27 (m, 7H, Ar-H), 5.41 (s, 1H, CHOH), 4.06 (t, 1H, *J* = 6.3 Hz, CH), 2.76 (d, 2H, -CH_2-), 2.66-2.19 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.93 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 183.3, 172.4,151.1, 146.0, 143.0,139.0, 130.4, 129.8, 129.2, 128.2, 117.1, 42.7, 35.1,32.9, 25.3; MS (ESI) m/z: 290.13 (M^+). *Anal*. Calcd. for $C_{20}H_{18}O_2$: C, 82.73; H, 6.25. Found: C, 82.71; H, 6.23%.

6-(hydroxymethylene)-8-(p-tolyl)-2,3,7,8-tetrahydro-1H-cyclopenta[b]naphthalen-5(6H)-one: (5b)

Color: Dark brown solid. Yield: 83.48 %. M.p.: 149-151 ^0C. IR (KBr, ν, cm^{-1}): 3391 (O-H), 2924-2853 (Ar-CH), 1713 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 14.23 (bs, 1H, OH vinyl), 7.97-7.17 (m, 6H, Ar-H), 5.40 (s, 1H, CHOH), 4.07 (t, 1H, *J* = 6.8 Hz, CH), 2.72 (d, 2H, -CH_2), 2.66-2.19 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 2.32 (s, 3H, CH_3), 1.93 (m, 2H), ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 183.1, 172.4, 151.1, 146.0, 140.0, 139.0, 135.8, 130.4, 129.8, 129.2, 128.0, 117.1, 42.7, 35.1, 32.9, 25.3, 21,3; MS (ESI) m/z: 304.15 (M^+). *Anal*. Calcd. for $C_{21}H_{20}O_2$: C, 82.86; H, 6.62. Found: C, 82.84; H, 6.61 %.

8-(4-fluorophenyl)-6-(hydroxymethylene)-2,3,7,8-tetrahydro-1H-cyclopenta[b]naphthalen- 5(6H)-one: (5c)

Color: Dark brown solid. Yield: 77.34 %. M.p.: 153-155 ^0C. IR (KBr, ν, cm^{-1}): 3341 (O-H), 2923 (Ar-CH), 1708 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 14.47 (bs, 1H, OH vinyl), 7.71-7.09 (m, 6H, Ar-H), 5.40 (s, 1H, CHOH), 4.06 (t, 1H, *J* = 6.1 Hz, CH), 2.72 (d, 2H, -CH_2-), 2.68-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 183.1, 172.1, 160.2, 151.1, 146.0, 139.0, 130.4, 129.8, 129.2, 128.0, 117.1, 116.0, 42.7, 35.1, 32.9, 25.3; MS (ESI) m/z: 308.12 (M^+). *Anal*. Calcd. for $C_{20}H_{17}FO_2$: C, 77.90; H, 5.56. Found: C, 77.88; H, 5.54 %.

6-(hydroxymethylene)-8-(4-nitrophynyl)-2,3,7,8-tetrahydro-1H-cyclopenta[b]naphthalen-5(6H)-one: (5d)

Color: Dark brown solid. Yield: 66.54 %. M.p.: 159-161 ^0C. IR (KBr, ν, cm^{-1}): 3392 (O-H), 2923-2853 (Ar-CH), 1681 (C=O); ^1H NMR ($CDCl_3$-400 MHz) δ ppm: 14.31 (bs, 1H, OH vinyl), 7.75-7.25 (m, 6H, Ar-H), 5.39 (s, 1H, CHOH), 4.09 (t, 1H, *J* = 6.0 Hz, CH), 2.71 (d, 2H, -CH_2-), 2.68-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 183.1, 172.1, 151.1, 149.1, 146.0, 145.4, 139.0, 130.4, 129.8, 129.0, 124.2, 117.1, 42.7, 35.1, 32.9, 25.3; MS (ESI) m/z: 335.12 (M^+). *Anal*. Calcd. for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11. Found: C, 71.61; H, 5.10 %.

2.1.5. General procedure for the synthesis of novel nitrogen containing analogues of podophyllotoxin 6a-d to 9a-d

A mixture of hydroxyl methylene tetralones (0.01 mol) 5a-d and hydrazine hydrate, hydroxyl amine, urea, thiourea (0.49 ml, 0.01 mol) in absolute ethanol was refluxed for 3 hrs. The excess of solvent was removed under reduced pressure. The solid obtained were collected and recrystallized from ethanol.

5-phenyl-2,4,5,7,8,9-hexahydroindeno[5,6-g]indozole: (6a)

Color: Dark brown solid. Yield: 71.37 %. M.p.: 142-144 ^{0}C. IR (KBr, ν, cm^{-1}): 3324 (N-H), 2925 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 12.28 (s, 1H, NH), 7.59-7.21 (m, 7H, Ar-H), 7.18 (s, 1H, pyrazole-CH), 4.35 (t, 1H, *J* = 6.3 Hz, CH), 3.29 (dd, 2H, *J* = 7.1 Hz, *J* = 7.3 Hz, CH_2), 2.68-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H) ; ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 145.3, 144.1, 143.0, 141.4 138.2, 133.1, 130.0, 129.2, 128.2, 126.3, 114.5, 46.0, 37.3, 33.2, 25.2, MS (ESI) m/z: 286.15 (M^+). *Anal*. Calcd. for $C_{20}H_{18}N_2$: C, 83.88; H, 6.34; N, 9.78. Found: C, 83.86; H, 6.32; N, 9.77 %.

5-phenyl-5,7,8,9-tetrahydro-4H-cyclopenta[6,7]naphtha[1,2-c]isoxazole: (6b)

Color: Dark brown solid. Yield: 66.30 %. M.p.: 144-145 ^{0}C. IR (KBr, ν, cm^{-1}): 2974 (Ar-CH), 1896 (isoxazole C-O) ; ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.43 (s, 1H, isoxazole-CH), 7.40-7.23 (m, 7H, Ar-H), 4.39 (t, 1H, *J* = 6.1 Hz, CH), 3.27 (dd, 2H, *J* = 7.8 Hz, *J* = 7.5 Hz, CH_2), 2.68-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 157.3, 154.7, 145.3, 143.0, 138.1, 130.1,129.2, 128.2,126.3, 126.0, 100.5, 46.0, 38.9, 33.2, 25.3; MS (ESI) m/z: 287.36 (M^+). *Anal*. Calcd. for $C_{20}H_{17}NO$: C, 83.59; H, 5.96; N, 4.87. Found: C, 83.55; H, 5.94; N, 4.85 %.

6-phenyl-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazolin-2-one: (6c)

Color: Dark brown solid. Yield: 71.50 %. M.p.: 152-154 ^{0}C. IR (KBr, ν, cm^{-1}): 3429-3255 (N-H), 2925 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.01(s, 1H, NH), 7.79-7.27 (m, 7H, Ar-H), 7.06 (s, 1H, quinazoline-CH), 4.07 (t, 1H, *J* = 6.4 Hz, Hz, CH), 3.11 (dd, 2H, *J* = 7.0 Hz, *J* = 7.4 Hz, CH_2); 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 164.5, 156.3, 147.5, 143.0, 138.3, 129.4, 129.2, 128.2, 128.0, 126.2, 120.7, 108.4, 42.0, 40.1, 33.0, 25.3; MS (ESI) m/z: 314.14 (M^+). *Anal*. Calcd. for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.21; H, 5.75; N, 8.89 %.

6-phynyl-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazoline-2-thione: (6d)

Color: Dark brown solid. Yield: 63.45 %. M.p.: 160-162 ^{0}C. IR (KBr, ν, cm^{-1}): 3369 (N-H), 2686 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 12.49 (s, 1H, NH), 7.68-7.27 (m, 7H, Ar-H), 5.23 (s, 1H, quinazoline-2-thione -CH), 4.38 (t, 1H, *J* = 6.0 Hz, CH), 2.89 (dd, 2H, *J* = 7.6 Hz, *J* = 7.3 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 180.4, 164.5, 147.5, 143.0, 141.1, 138.3, 129.4, 129.2, 128.2, 126.2, 120.7, 106.4, 42.5, 40.1, 33.0, 25.3; MS (ESI) m/z: 330.12 (M^+). *Anal*. Calcd. for $C_{21}H_{18}N_2S$: C, 76.33; H, 5.49; N, 8.48. Found: C, 76.31; H, 5.47; N, 8.46 %.

5-(p-tolyl)-2,4,5,7,8,9-hexahydroindeno[5,6-g]indozole: (7a)

Color: Dark brown solid. Yield: 72.36 %. M.p.: 141-143 ^{0}C. IR (KBr, ν, cm^{-1}): 3210 (N-H), 2922 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 11.89 (s, 1H, NH), 7.61 (s, 1H, pyrazole-CH), 7.43-7.17 (m, 6H, Ar-H), 4.35 (t, 1H, *J* = 6.3 Hz, CH), 3.29 (dd, 2H, *J* = 7.1 Hz, *J* = 7.3 Hz, CH_2); 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 2.31 (s, 3H, CH_3), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 145.2, 144.1, 140.0, 138.1, 136.0, 133.1, 130.1, 129.5, 128.1, 114.6, 46.0, 37.1, 33.2, 25.3, 21.3; MS (ESI) m/z: 300.16 (M^+). *Anal*. Calcd. for $C_{21}H_{20}N_2$: C, 83.96; H, 6.71; N, 9.33. Found: C, 83.94; H, 6.70; N, 9.31 %.

5-(p-tolyl)-5,7,8,9-tetrahydro-4h-cyclopenta[6,7]naphtha[1,2-c]isoxazole: (7b)

Color: Dark brown solid. Yield: 65.26 %. M.p.: 143-145 ^{0}C. IR (KBr, ν, cm^{-1}): 2921 (Ar-CH),1897 (isoxazole C-O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.45 (s, 1H, isoxazole-CH), 7.43-7.17 (m, 6H, Ar-H), 4.39 (t, 1H, *J* = 6.1 Hz, CH), 3.27-3.04 (dd, 2H, *J* = 7.8 Hz, *J* = 7.5 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 2.31 (s, 3H, CH_3), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 157.3, 154.6, 145.2, 140.0, 138.1, 136.0, 130.1, 129.5, 128.1, 100.6, 46.0, 38.9, 33.2, 25.3, 21.5; MS (ESI) m/z: 301.15 (M^+). *Anal*. Calcd. for $C_{21}H_{19}NO$: C, 83.69; H, 6.35; N, 4.65. Found: C, 83.67; H, 6.33; N, 4.63 %.

6-(p-tolyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazolin-2-one: (7c)

Color: Dark brown solid. Yield: 70.46 %. M.p.: 153-155 ^{0}C. IR (KBr, ν, cm^{-1}): 3430-3335 (N-H), 2923 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.13 (s, 1H, NH), 7.79-7.27 (m, 6H, Ar-H), 7.06 (s, 1H, quinazolin-CH), 4.01 (t, 1H, *J* = 6.4 Hz, Hz, CH), 3.27 (dd, 2H, *J* = 7.0 Hz, *J* = 7.4 Hz, CH_2); 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 2.31 (s, 3H, CH_3), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 164.5, 156.3, 147.1, 140.0, 138.3, 136.0, 129.5, 129.2, 128.1, 128.0, 120.7, 108.4, 42.7, 40.1, 33.0, 25.3, 21.3; MS (ESI) m/z: 328.16 (M^+). *Anal*. Calcd. for $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53. Found: C, 80.44; H, 6.12; N, 8.51 %.

6-(p-tolyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazoline-2-thione: 7d:

Color: Dark brown solid. Yield: 61.35 %. M.p.: 159-161 ^{0}C. IR (KBr, ν, cm^{-1}): 3369 (N-H), 3154-2685 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 12.49 (s, 1H, NH), 7.78-7.23 (m, 6H, Ar-H), 5.23 (s, 1H, quinazolin-CH), 4.08 (t, 1H, *J* = 6.0 Hz, CH), 3.29 (dd, 2H, *J* = 7.6 Hz, *J* = 7.3 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 2.31 (s, 3H, CH_3), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 184.4, 164.5, 147.1, 140.0, 138.3, 136.0, 129.5, 129.2, 128.1, 120.7, 106.4, 42.7, 40.1, 33.0, 25.3, 21.3; MS (ESI) m/z: 344.13 (M^+). *Anal*. Calcd. for $C_{22}H_{20}N_2S$: C, 76.71; H, 5.85; N, 8.13. Found: C, 76.70; H, 5.83; N, 8.11 %.

5-(4-fluorophenyl)-2,4,5,7,8,9-hexahydroindeno[5,6-g]indazole: 8a:

Color: Dark brown solid. Yield: 72.36 %. M.p.: 141-143 ^{0}C. IR (KBr, ν, cm^{-1}): 3276 (N-H), 2853 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 11.78 (s, 1H, NH), 7.59-7.19 (m, 6H, Ar-H), 7.01 (s, 1H, pyrazole-CH), 4.35 (t, 1H, *J* = 6.3 Hz, CH), 3.26 (dd, 2H, *J* = 7.1 Hz, *J* = 7.3 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 160.3, 145.3, 144.1, 138.6, 138.1, 133.1, 130.1, 129.7, 126.3, 116.0, 114.5, 46.0, 37.2, 33.2, 25.2; MS (ESI) m/z: 304.14 (M^+). *Anal*. Calcd. for $C_{20}H_{17}FN_2$: C, 78.92; H, 5.63; N, 9.20. Found: C, 78.90; H, 5.61; N, 9.18 %.

5-(4-fluorophynyl)-5,7,8,9-tetrahydro-4H-cyclopenta[6,7]naphtha[1,2-c]isoxazole: 8b:

Color: Dark brown solid. Yield: 65.26 %. M.p.: 143-145 ^{0}C. IR (KBr, ν, cm^{-1}): 2922 (Ar-CH); 1603 (isoxazole C-O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.45 (s, 1H, isoxazole-CH), 7.53-7.19 (m, 6H, Ar-H), 4.35 (t, 1H, *J* = 6.1 Hz, CH), 3.27 (dd, 2H, *J* = 7.8 Hz, *J* = 7.5 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 160.3, 154.7, 154.3, 145.3, 138.6, 138.1, 130.1, 129.7, 126.3, 116.0, 100.5, 46.0, 38.2, 33.2, 25.2; MS (ESI) m/z: 305.12 (M^+). *Anal*. Calcd. for $C_{20}H_{16}FNO$: C, 78.69; H, 5.28; N, 4.59. Found: C, 78.67; H, 5.26; N, 4.57 %.

6-(4-fluorophynyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazolin-2-one: 8c:

Color: Dark brown solid. Yield: 70.46 %. M.p.: 153-155 ^{0}C. IR (KBr, ν, cm^{-1}): 3430-3336 (N-H), 2923 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.05 (s, 1H, NH), 7.79-7.20 (m, 6H, Ar-H), 7.06 (s, 1H,quinazolin-CH), 4.07 (t, 1H, *J* = 6.4 Hz, Hz, CH), 2.83 (dd, 2H, *J* = 7.0 Hz, *J* = 7.4 Hz, CH_2) 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.93 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 164.5, 160.3, 156.3, 145.8, 138.6, 138.1, 129.7, 129.4, 128.1, 120.6, 116.0, 108.3, 42.0, 40.1, 33.2, 25.2; MS (ESI) m/z: 332.37 (M^+). *Anal*. Calcd. for $C_{21}H_{17}N_2O$: C, 75.89; H, 5.16; N, 8.43. Found: C, 75.87; H, 5.14; N, 8.41 %.

6-(4-fluorophynyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazoline-2-thione: 8d:

Color: Dark brown solid. Yield: 61.35 %. M.p.: 159-161 ^{0}C. IR (KBr, ν, cm^{-1}): 3369-3269 (N-H), 2924-2686 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 12.49 (s, 1H, NH), 7.78-7.23 (m, 6H, Ar-H), 5.23 (s, 1H, qinazolin-CH), 4.08 (t, 1H, *J* = 6.0 Hz, CH), 3.19 (dd, 2H, *J* = 7.6 Hz, *J* = 7.3 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.93 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 180.3, 164.5, 160.2, 147.5, 141.1, 138.5, 138.1, 129.8, 129.1, 120.4, 116.0, 106.5, 42.7, 40.1, 32.7, 25.2; MS (ESI) m/z: 348.11 (M^+). *Anal*. Calcd. for $C_{21}H_{17}FN_2S$: C, 72.39; H, 4.92; N, 8.04. Found: C, 72.37; H, 4.90; N, 8.02 %.

5-(4-nitrophynyl)-2,4,5,7,8,9-hexahydroindeno[5,6-g]indazole 9a:

Color: Dark brown solid. Yield: 72.36 %. M.p.: 141-143 ^{0}C. IR (KBr, ν, cm^{-1}): 3222 (N-H), 2922-2853 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 11.98 (s, 1H, NH), 8.19-7.31 (m, 6H, Ar-H), 7.21 (s, 1H, indazole-CH), 4.37 (t, 1H, *J* = 6.3 Hz, CH), 3.26 (dd, 2H, *J* = 7.1 Hz, *J* = 7.3 Hz, CH_2); 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.93 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 149.1, 145.2, 145.0, 144.1, 138.1, 132.1, 130.1, 129.2, 126.3, 124.2, 114.6, 46.0, 37.2, 33.2, 25.3 ; MS (ESI) m/z: 331.13 (M^+). *Anal*. Calcd. for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.47; H, 5.15; N, 12.66 %.

5-(4-nitrophynyl)-5.7,8,9-tetrahydro-4H-cyclopenta[6,7]naphtha[1,2-c]isoxazole: 9b:

Color: Dark brown solid. Yield: 65.26 %. M.p.: 143-145 ^{0}C. IR (KBr, ν, cm^{-1}): 2921-2666 (Ar-CH); 1577 (isoxazole C-O); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.45 (s, 1H, isoxazole-CH), 8.23-7.19 (m, 6H, Ar-H), 4.35 (t, 1H, *J* = 6.1 Hz, CH), 3.27 (dd, 2H, *J* = 7.8 Hz, *J* = 7.5 Hz, CH_2), 2.70-2.20 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.92 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 157.3, 154.5, 149.1, 145.3, 145.1, 138.1, 130.1, 129.1, 126.3, 124.4, 100.5, 46.0, 38.9, 33.2, 25.2; MS (ESI) m/z: 332.12 (M^+). *Anal*. Calcd. for $C_{20}H_{16}N_2O_3$: C, 72.28; H, 4.85; N, 8.43. Found: C, 72.26; H, 4.83; N, 8.41 %.

6-(4-nitrophynyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazolin-2-one: 9c:

Color: Dark brown solid. Yield: 70.46 %. M.p.: 153-155 ^{0}C. IR (KBr, ν, cm^{-1}): 3429-3333 (N-H), 2923-2853 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 8.04 (s, 1H, NH), 7.89-7.20 (m, 6H, Ar-H), 7.06 (s, 1H,quinazolin-2-one-CH), 4.07 (t, 1H, *J* = 6.4 Hz, Hz, CH), 2.83 (dd, 2H, *J* = 7.0 Hz, *J* = 7.4 Hz, CH_2) 2.70-2.25 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.94 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 164.5, 156.3, 149.1, 147.6, 145.4, 138.2, 129.4, 129.1, 128.1, 124.3, 120.7, 108.3, 42.7, 40.1, 32.8,

25.2; MS (ESI) m/z: 359.13 (M^+). *Anal*. Calcd. for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.16; H, 4.75; N, 11.67 %.

6-(4-nitrophynyl)-3,5,6,8,9,10-hexahydro-2H-indeno[5,6-h]quinazoline-2-thione: 9d:

Color: Dark brown solid. Yield: 61.35 %. M.p.: 159-161 ^{0}C. IR (KBr, ν, cm^{-1}): 3274-3159 (N-H), 2921-2682 (Ar-CH); ^{1}H NMR ($CDCl_3$-400 MHz) δ ppm: 12.49 (s, 1H, NH), 7.98-7.23 (m, 6H, Ar-H), 5.24 (s, 1H, quinazoline-2-thione-CH), 4.08 (t, 1H, *J* = 6.0 Hz, CH), 2.83 (dd, 2H, *J* = 7.0 Hz, *J* = 7.4 Hz, CH_2) 2.70-2.25 (tt, 4H, *J* = 5.9 Hz, *J* = 6.6 Hz, CH_2), 1.94 (m, 2H); ^{13}C NMR ($CDCl_3$-100 MHz) δ ppm: 180.3, 164.5, 149.1, 147.5, 145.8, 145.4, 141.1, 138.3, 129.4, 129.1, 124.3, 120.6, 106.1, 42.7, 40.2, 32.9, 25.3; MS (ESI) m/z: 375.10 (M^+). *Anal*. Calcd. for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19. Found: C, 67.16; H, 4.54; N, 11.17 %.

2.2. Antimitotic studies

The antimitotic activity of synthesized novel nitrogen containing analogues of podophyllotoxin **6a-d to 9a-d** was examined using onion root tip method and the ID_{50} 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 (100, 200 and 300 ppm). To study the effect of novel nitrogen containing 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 compounds (7x3) after removing the old root from it and immersion for 24 hrs. intervals respectively 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 hrs. After 24 hrs. 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 hr. The roots were taken on a clean glass slide and squashed using 45 % acetic acid following the method of Levan [8]. 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 shield with molten paraffin wax and slide was observed under microscope. Mitotic Index(M.I) was calculated by following method of Fissceja [9]. The mitotic index was determined by examination minimum of zone cells. Three replicates were made for each calculation. The slides were observed under microscope and photographed.

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

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 300 ppm 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 nitrogen containing analogues of podophyllotoxin for antimitotic activity were calculated individually [10].

2.3. Antimicrobial studies

2.3.1. Antibacterial activity

The antimicrobial assay was performed by agar disc diffusion method [11, 12]. For antibacterial activity, the molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 µl of the inoculum (1 x 10^8 Cfu) and poured into the sterile Petri plates (Himedia). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of 10.0 mg/ml of the test compound in the dimethylformamide (DMF), allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ^{0}C for 24 hrs. Antibacterial activity of all the novel nitrogen containing analogues of podophyllotoxin was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). The medium with dimethylformamide (DMF) as solvent was used as a negative control whereas media with Chloromphenicol (standard antibacterial drug) was used as positive control. The experiments were performed in triplicates.

2.3.2. Antifungal activity

Novel nitrogen containing analogues of podophyllotoxin were dissolved in dimethylformamide (DMF) and evaluated for their antifungal activity by disc diffusion method. For agar disc diffusion method, one week old culture of the mold was used as inoculums for evaluating antifungal activity of chemical compounds. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 µl of the inoculum (1 x 10^8 Cfu) and poured into the sterile Petri plates (Himedia) and the disc (0.7cm) (Hi-Media) was saturated with 100 µl of 10.0 mg/ml of the test compound in the dimethylformamide (DMF), allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 25 ^{0}C for 7 days. Antifungal

activity of all the novel nitrogen containing analogues of podophyllotoxin was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). The medium with dimethylformamide (DMF) as solvent was used as a negative control where as media with Nystatin (standard antifungal drug) were used as positive control. The experiments were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthesis of novel nitrogen and oxygen containing heterocyclic analogues of podophyllotoxin has been carried out by chalcone route scheme 1. The benzylideneacetophenones (chalcones) 2a-d were prepared in high yields by Claisen-Schmidt reaction of acetophenones 1a with benzaldehyde,4-methyl benzaldehyde, 4-fluoro benzaldehyde and 4- nitro benzaldehyde in the presence of sodium hydroxide in water-ethanol mixture [11-14]. The structures of the chalcones were confirmed by IR and ^{1}H NMR spectral studies. IR spectra of compounds 2a-d showed the C=C stretching frequency in the range 1626-1613 cm^{-1} and ^{1}H NMR showed the absence of aldehyde proton at 9.83 ppm. The cyclopropyl ketones 3a-d were prepared in good yields by the reaction of chalcones 2a-d with trimethylsulfoxonium iodide (TMSOI) in the presence of sodium hydride in dry DMSO [15, 16]. The sodium hydride acts as a base which abstracts a proton from the methyl group in trimethylsulfoxonium iodide to form a dimethylsulfoxonium methylide. It attacks nucleophilically the β-carbon atom of the chalcone which acts as Michael receptors to form an enolate ion, which undergoes nucleophilic attack on the methylene carbon atom bearing the dimethylsulfoxonium cation intramolecularly finally to form the desired cyclopropyl ketones. The structures of compounds 3a-d were confirmed by IR spectra. The IR spectra showed the C=O stretching band in the range 1687-1671 cm^{-1} and ^{1}H NMR showed the cyclopropane CH and CH_2 peak at the range 2.21-2.00 and 0.83-0.78 ppm respectively. Tetralones 4a-d were prepared in good yields by the Friedel-Craft's intramolecular cyclization reaction of cyclopropyl ketones 3a-d in the presence of anhyd. stannic chloride and acetic anhydride in dry dichloromethane [15-17]. The cyclopropyl ketones undergoes electrophilic ring opening in the presence of Lewis acid to give benzylcarbocationic intermediate which is intramolecularly attacked by aryl ring π-electrons resulting in the formation of a six membered ring with a pendant carbocation. This readily gives up proton to form tetralones. Acetic anhydride which facilitates the formation of desired tetralones. In its IR spectra appeared absorption bands in the range 3133-2934 cm^{-1} and 1705-1685 cm^{-1} corresponds respectively to C-H and C=O stretching frequencies and ^{1}H NMR of the ring B proton appears in the range 2.65-2.18 ppm. They are key intermediates for the synthesis of the novel nitrogen containing tetrolone analogues of podophyllotoxin. The tetralones on formylation to give substituted hydroxylmethylene tetralones 5a-d [18]. Formylation of the presently synthesized tetralones with ethyl formate using sodium hydride as the base at room temperature expected products in good yields. The β-dicarbonyl compound which exists in the enol form show the carbonyl absorption in the region 1640 -1610 cm^{-1}. Generally 1, 3-diketones absorption peak with high intensity at 1715 cm^{-1}. The NMR absorption peak appears at very down field (i.e., around δ 14.5) is attributed to the intramolecular hydrogen bonding figure 2.

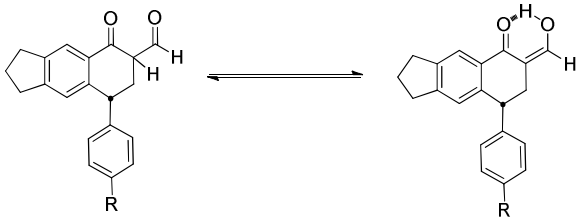
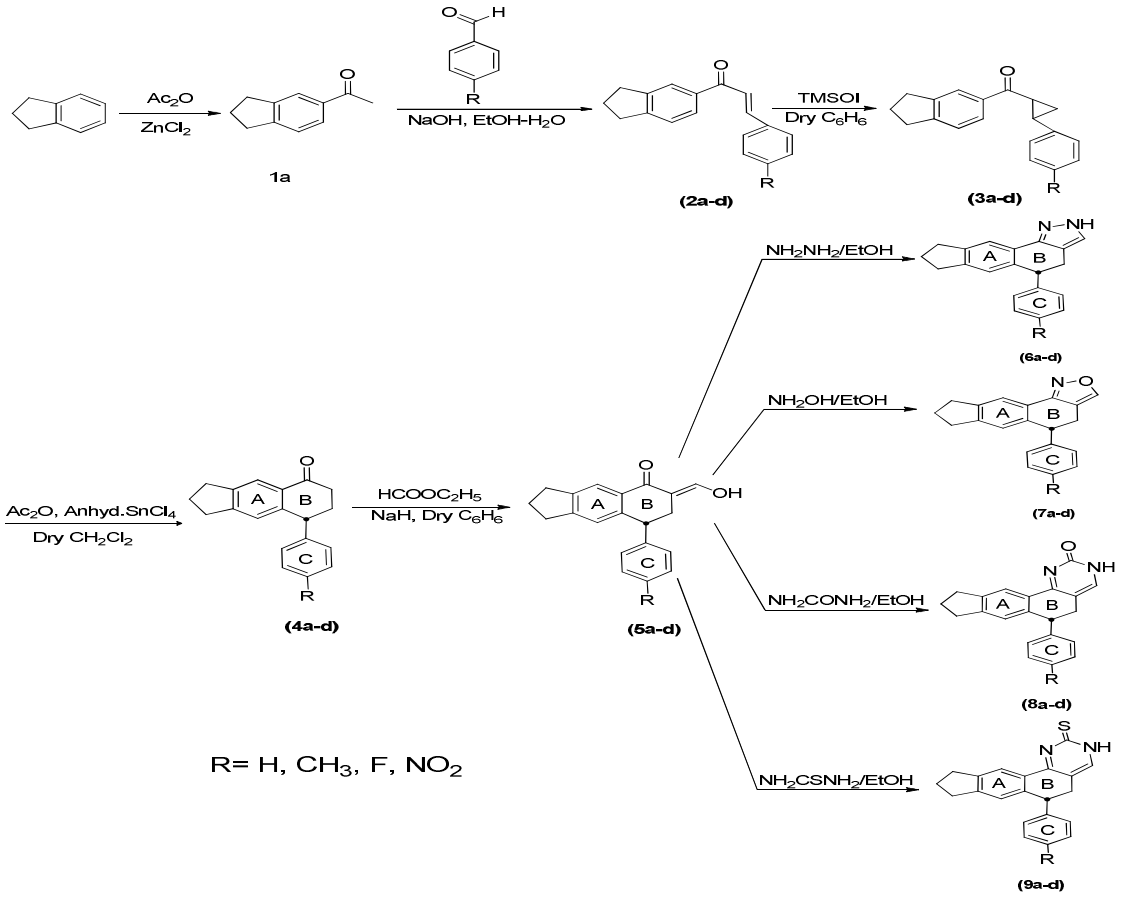


Figure - 2: β-dicarbonyl compound which exist in the enol form.

At the same time, the true alcoholic hydroxyl band near 3610 cm^{-1}is absent in enols, but there is a band near 3258-3251 cm^{-1} (s) which is attributed to the chelated hydroxyl group. ^{1}H NMR showed the vinylic proton absorption at δ 14.80-15.00. The novel nitrogen containing analogues of podophyllotoxin 6a-d to 9a-d were synthesized in high yields by the condensation of hydroxylmethylene tetralones and hydrazine hydrate, hydroxyl amine hydrochloride, urea and thiourea in absolute ethanol [19]. The products were purified by recrystallization from ethanol and column chromatography by using benzene-ethyl acetate mixture as eluent and silica gel as an adsorbent. The compounds 6a-d exhibited NH stretching band at 3410-3350 cm^{-1} and proton NMR showed singlet NH peak at 12.58-12.99 ppm. Based on this, the synthesized compounds were confirmed.

3.2. Antimitotic activity

Allium cepa has been used to evaluate the antimitotic activity of novel nitrogen containing tetralone analogues of podophyllotoxin. Root tip cells in 6a-d to 9a-d exhibited changes in cellular morphology such as slight elongation in shape with many of them remain in the earliest stages of mitosis called prophase stage. Onion roots in



Scheme - 1: Protocol for synthesis of novel nitrogen containing tetralone analogues of podophyllotoxin 6a-d to 9a-d.

compound 6a-d to 9a-d of 100, 200 and 300 ppm at 24 hrs exhibited changes in chromosomes and shape of the cells with elongated appearance. Using cytotoxic nature of novel nitrogen containing tetralone analogues of podophyllotoxin showed very less number of dividing cells. Change in chromosomes and cellular morphology were achieved in increasing time and concentration. Treatment of root meristem with compound 6a-d to 9a-d exhibited less change in cell shape with elongated appearance. Compounds 6a-d to 9a-d of 100, 200 and 300 ppm were used for this experiment, nitrogen containing heterocyclic ring present in 6a-d to 9a-d compounds showed highest antimitotic activity.

The result showed at 24 hrs., the percentage of inhibition increases than at 12 and 18 hrs compared to control. Meanwhile at 300 ppm, the percentage of inhibition of the germinated root tips in 6a-d to 9a-d compounds little above the value at 24 hrs. but the percentage of inhibition was dropped significantly below 24 hrs. Table 1 shows the effect of concentration and time on the cell division of *Allium cepa*. The result shows that the percentage of inhibition of compound 7b was significantly highest with 300 ppm at 24 hrs, compound 7a,7c, and 9b showed least inhibition while others exhibited moderate inhibition compared to control.

Figure - 3: A; Anaphase and B; Metaphase

From above observations, it can be seen that partial-c-mitosis, full-c-mitosis with partially functional spindles and comparatively normal mitotic cells phases, chromosomal bridge and chromosomal breakage were noticed in various cells of the same root tip between 24 hrs time duration figure 3: A and B. Therefore antimitotic ability of novel nitrogen containing tetralone analogues of podophyllotoxin was remarkable in controlling the cell division and hence acts as a very good antimitotic agent. The results and graphical representation of antimitotic activity are given in table 1 and figure 4.

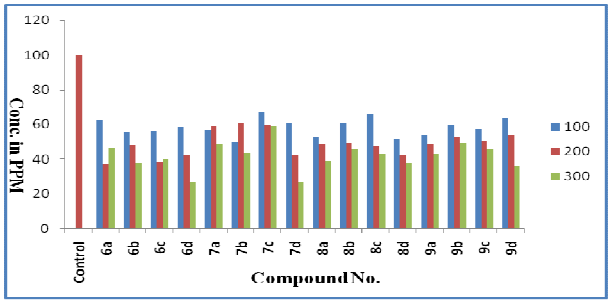


Figure - 4: Graphical representation of anti mitotic activity of synthesized compounds 6a-d to 9a-d by onion root tip method.

Table - 1: Antimitotic activity of the compounds 6a-d to 9a-d by onion root tip method

Compound No.	Concentrations In PPM	Percentage of dividing cells	Percentage of dividing cells compare to control	Percentage of inhibition compare to control	Average	ID_{50} in ppm
Control		58.95				
6a	100 ppm	26.19	44.42	55.57	62.17	145
		18.49	31.36	68.63		
		22.22	37.69	62.31		
	200 ppm	39.58	67.14	32.86	37.36	
		33.33	56.53	43.47		
		37.88	64.25	35.75		
	300 ppm	30.00	50.89	49.11	46.56	
		33.36	56.59	43.41		
		31.14	52.82	47.18		
6b	100 ppm	23.12	39.21	60.79	55.49	155
		26.35	44.76	55.24		
		29.21	49.55	50.45		
	200 ppm	32.32	54.82	45.18	48.00	
		33.71	57.14	42.86		
		25.96	44.03	55.98		
	300 ppm	31.81	53.96	46.04	37.82	
		39.97	67.80	32.29		
		38.23	64.85	35.15		
6c	100 ppm	37.56	51.71	48.29	56.31	135
		35.20	45.71	54.29		
		31.62	33.63	66.37		
	200 ppm	37.74	64.02	35.96	38.67	
		33.93	57.55	42.45		
		36.78	62.39	37.61		
	300 ppm	44.72	75.86	24.11	39.85	
		35.29	59.86	40.14		
		26.35	44.69	55.31		
6d	100 ppm	29.19	49.51	50.49	58.37	145
		22.86	38.77	61.23		
		21.58	36.60	63.41		
	200 ppm	33.81	57.35	42.65	42.07	
		29.18	49.49	50.51		
		39.47	66.95	33.05		

		39.78	67.48	32.52		
	300 ppm	48.86	82.88	17.12	26.69	
		41.00	69.55	30.45		
Control		48.84				
		21.65	44.32	55.68		
	100 ppm	18.12	37.10	62.90	56.98	
		23.26	47.62	52.38		
		21.15	43.30	56.70		
7a	200 ppm	13.02	26.65	73.35	59.38	250
		24.96	51.10	48.09		
		21.65	44.32	55.68		
	300 ppm	28.12	57.57	42.43	48.26	
		26.05	53.33	46.67		
		18.15	37.16	62.84		
	100 ppm	28.32	57.98	42.02	49.96	
		26.85	54.97	45.03		
		12.82	26.24	73.76		
7b	200 ppm	28.49	58.33	41.67	60.85	100
		16.05	32.86	67.14		
		31.11	63.69	36.31		
	300 ppm	22.02	45.08	54.92	43.66	
		29.42	60.23	39.77		
		37.30	23.63	76.37		
	100 ppm	22.82	53.28	46.72	67.04	
		38.12	21.95	78.05		
		37.70	22.81	77.19		
7c	200 ppm	28.54	41.57	58.43	60.03	290
		36.41	45.46	44.54		
		27.40	43.90	56.10		
	300 ppm	32.86	32.72	67.28	59.13	
		38.62	45.99	54.01		
		27.31	44.09	55.91		
	100 ppm	33.21	32.01	67.99	60.48	
		28.11	42.45	57.55		
		41.81	85.60	53.18		
7d	200 ppm	38.43	78.68	39.32	42.45	165
		26.52	54.38	34.85		
		22.87	46.82	14.40		
	300 ppm	29.64	60.68	21.32	27.11	
		31.82	65.15	45.61		
Control		59.84				
		32.00	53.47	50.62		
	100 ppm	28.12	46.19	62.69	52.55	
		32.22	53.84	44.35		
		29.55	49.38	46.53		
8a	200 ppm	22.33	37.31	53.80	48.83	140
		33.30	55.64	46.16		
		39.21	65.52	34.48		
	300 ppm	31.62	52.84	47.16	39.18	
		21.50	64.08	35.92		

		22.69	37.91	62.09		
	100 ppm	28.39	47.44	52.56	60.60	
		39.22	34.46	65.54		
		32.32	54.01	45.99		
8b	200 ppm	33.71	55.68	44.31	48.97	185
		25.96	43.38	56.62		
		36.58	61.12	38.87		
	300 ppm	32.62	54.51	45.49	45.72	
		28.23	47.17	52.82		
		47.61	20.44	79.56		
	100 ppm	32.20	46.19	53.81	65.63	
		38.02	36.46	63.53		
		37.84	62.70	37.30		
8c	200 ppm	38.36	45.08	54.92	47.72	185
		26.55	49.04	50.96		
		37.52	63.23	36.77		
	300 ppm	26.98	64.10	35.84	42.75	
		29.35	44.36	55.64		
		34.91	41.67	58.33		
	100 ppm	26.69	55.39	44.60	51.87	
		31.54	47.29	52.70		
		29.81	49.89	50.10		
8d	200 ppm	38.78	63.90	36.09	42.14	115
		42.69	59.97	40.23		
		29.86	49.81	50.18		
	300 ppm	38.24	64.80	35.19	38.00	
		35.89	71.34	28.65		
Control		69.15				
		42.21	48.08	51.92		
	100 ppm	38.87	47.18	52.82	53.81	
		43.65	43.29	56.71		
		33.25	61.04	39.01		
9a	200 ppm	32.63	56.21	43.78	48.89	165
		29.94	63.12	63.88		
		34.69	50.16	49.84		
	300 ppm	39.23	56.72	43.28	42.68	
		45.85	65.07	34.93		
		22.67	32.78	67.22		
	100 ppm	29.91	43.25	56.75	60.01	
		28.52	41.24	58.76		
		32.23	46.60	53.40		
9b	200 ppm	29.87	43.19	56.81	52.73	220
		35.96	52.00	47.98		
		33.78	51.20	48.85		
	300 ppm	29.82	56.88	43.12	49.08	
		38.23	44.73	55.27		
		27.61	39.92	60.08		
	100 ppm	22.44	32.45	67.55	57.55	
9c		38.02	54.98	45.02		200
	200 ppm	33.64	49.48	50.52	50.43	
		40.36	53.18	46.82		

		38.75	46.05	53.95		
		34.22	48.64	51.64		
	300 ppm	36.78	58.36	41.64	45.75	
		31.85	56.03	43.97		
		24.11	34.85	65.15		
	100 ppm	29.25	42.29	57.74	63.38	
		23.14	33.46	66.54		
		29.41	42.53	57.47		
9d	200 ppm	31.54	45.61	54.39	53.67	200
		35.15	50.83	49.17		
		22.00	68.81	31.81		
	300 ppm	28.14	59.31	40.69	36.30	
		25.18	63.59	36.41		

Table - 2: Antibacterial activity of the compounds (6a–d to 9a-d). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Compound No.	*V. Parahaemolyticus* gram negative	*V. Cholera* Gram negative	*E. Coli* gram negative	*S. Sonnei* gram negative	*S. Typhi* gram negative	*S. Aureus gram positive*
Conc. in mg/ml	10.0	10.0	10.0	10.0	10.0	10.0
Chloramphenicol	38.4±0.08	40.8±0.06	35.0±0.04	45.6±0.06	30.0±0.03	36.0±0.20
6a	0	0	0	0	0	0
6b	35.0±0.05	36.0±0.25	33.8±0.10	40.0±0.20	0	0
6c	0	0	0	0	0	0
6d	0	0	0	0	0	0
7a	0	0	0	0	0	0
7b	26.1±0.03	27.4±0.25	26.6±0.04	37.0±0.25	0	0
7c	0	0	0	0	0	0
7d	0	0	0	0	0	0
8a	0	0	0	0	0	0
8b	22.2±0.06	24.5±0.08	25.6±0.20	40.6±0.21	0	0
8c	0	0	0	0	0	0
8d	0	0	0	0	0	0
9a	0	0	0	0	0	0
9b	37.8±0.08	39.4±0.06	33.4±0.08	40.6±0.08	29.0±0.10	35.0±0.04
9c	0	0	0	0	0	0
9d	0	0	0	0	0	0

Values are Mean of triplicates Standard 10 mg/disc

3.3. Antimicrobial activity

The synthesized analogues 6a-d to 9a-d were also screened for antibacterial activity studies at a concentration of 10 mg/ml using DMF as a control against *V. Parahaemolyticus*, *S. Sonnei, E. Coli, V. Cholera, S. Typhi* and *S. Aureus;* by disc diffusion method on nutrient agar media. Chloromphenicol was used as standard drug. The antifungal activity of the compounds was evaluated against *F. Verticillioides* and *A. Niger* at the concentration of 10 mg/ml using Nystatin as standard drug. Dimethylformamide (DMF) was used as negative control. The antimicrobial activity data is reported in table 2 and 3. In the antimicrobial activity, among the synthesized analogues, compound 6b, 7b, 8b and 9b possessing tolyl substituent present at ring A and C showed high activity against all the bacterial and fungal strains. But other Compounds not show activity against all antibacterial strains and also against *A. Niger* fungal strain but least against *F. Verticillioides* fungal strain. Compound 6b, 7b, 8b and 9b possessing tolyl on ring A and C ring showed high activity against *S. Sonnei* bacterial strain and exhibited least activity against

remaining bacterial and fungal strains. Remaining compounds having fluorine and nitro substituent present at ring C compound exhibited least activity against all the bacterial and fungal strains.

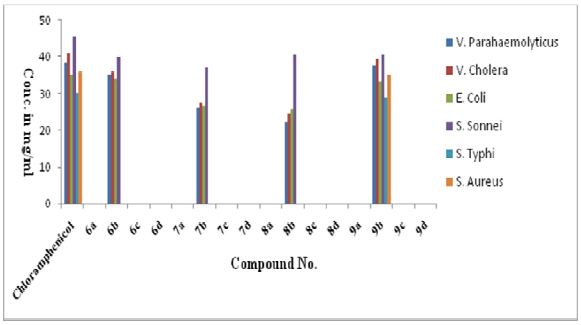


Figure - 5: Graphical representation of antibacterial activity of the compounds 6a-d to 9a-d synthesized compounds against tested bacterial strains by disc diffusion method.

The human race is always been in war with the bacteria, though bacterial resistance against antibiotics has been recognized almost since the dawn of antibiotic era, the increase in emergence of antibiotic-resistant bacteria is being observed from the past several decades (Fair and Tor, 2014). Antibiotics are special class of therapeutics; in which medicinal chemistry have great challenges to bring new molecule centered approach to fight against existing bacterial targets. In an effort to evaluate sixteen new synthetic compounds; mainly with para positions replacement with phenyl, p – tolyl, 4 – Fluro phenyl and *4* – nitro phenyl and also with similar additional groups against six human infectious bacteria viz. *V. Parahaemolyticus*, *S. Sonnei, E. Coli, V. Cholera, S. Typhi* and *S. Aureus;* The studied enabled in finding potential antimicrobial agents at the preliminary screening by agar diffusion method (Fairbrother and Martyn, 1951). Results recorded during evaluation (Table 2 and Fig. 5) of anti-microbial potentials of synthetic compounds suggests that, synthetic compounds with *p* – tolyl group at para position have proved to be potent to inhibit the growth of tested isolates. However, among the four *p*–tolyl derivatives (6b, 7b, 8b and 9b) 9b with the hexa hydro – 2 – h indeno (5, 4 – h) quinozoline – 2 – thione proved to be an potential antibacterial agent against all the six tested isolates when compared with 8b, 9b and 10b which failed to inhibit the growth of *S. Typhi* and *S. Aureus.* In this study, among the sixteen synthetic compounds only p – tolyl substitutes exhibited the antimicrobial potential when compared with 4 – Fluro phenyl, 4 – Nitro phenyl and Phenyl groups at the para position.

With this experimental proof, study suggests that the synthetics with *p* – tolyl group will be an potential antibacterial agent, and need to be further assessed to understand the toxicity and its interactions with biological which will surely benefit the pharmaceuticals to develop the potential antibacterial agents against different infectious targets.

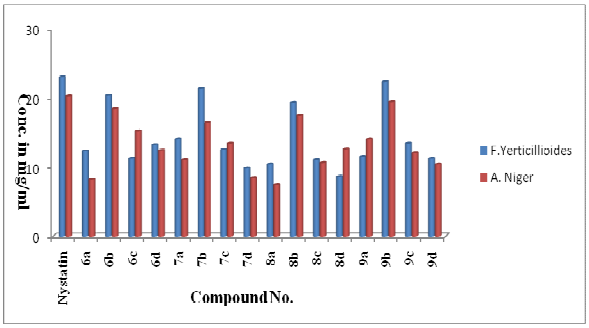


Figure - 6: Graphical representation of antifungal activity of the compounds 6a-d to 9a-d synthesized compounds against tested bacterial strains by disc diffusion method.

Table - 3: Antifungal activity of the compounds 6a–d.to 9a-d Inhibitory zone (diameter) mm of the synthesized compounds against tested fungal strains by disc diffusion method

Compound No.	*F.verticillioides*	*A. niger*
Conc.in mg/ml	10.0	10.0
Nystatin	23.0±00.4	20.2±0.02
6a	12.3±0.04	08.2±0.04
6b	20.3±0.08	18.4±0.06
6c	11.2±0.06	15.2±0.02
6d	13.2±0.03	12.4±0.02
7a	14.0±004	11.0±0.08
7b	21.3±0.08	16.4±0.06
7c	12.5±0.25	13.4±0.04
7d	09.8±0.07	08.4±0.02
8a	10.4±004	07.4±0.06
8b	19.3±0.04	17.4±0.06
8c	11.0±0.08	10.6±0.25
8d	08.6±0.04	12.6±0.04
9a	11.5±0.05	14.0±0.02
9b	22.3±0.08	19.4±0.08
9c	13.4±0.07	12.0±0.03
9d	11.2±0.04	10.4±0.04

Values are Mean of triplicates Standard 10 mg/disc

4. CONCLUSION

In conclusion, we have reported a convenient protocol for the synthesis of novel nitrogen containing analogues of podophyllotoxin by chalcone route. The antimitotic and antimicrobial property was evaluated using *in vitro* models. The novel nitrogen containing analogues of podophyllotoxin having nitrogen containing heterocyclic ring and tolyl group was found to be the most effective when compared to standard drugs (Chloromphenicol and Nystatin). All the synthesized novel nitrogen containing analogues of podophyllotoxin 6a-d to 9a-d were identified as antimitotic and antimicrobials. For antimitotic activity, among the synthesized analogues, compound 7b and 8d showed more inhibitions and the compounds 6b, 7b, 8b and 9b exhibited high antibacterial activity against all the bacterial and antifungal strains. It is conceivable from these studies that introducing of nitrogen containing heterocyclic ring moiety to key intermediate tetralones can exhibit interesting antimitotic and antimicrobial properties. The novel nitrogen containing analogues of podophyllotoxin may be useful in the pharmaceutical and medicinal field.

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