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Synthesis and Pharmacological Studies of New Pyrazole Analogues of Podophyllotoxin[1]

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Abstract—The pyrazole analogues of podophyllotoxin were synthesized by the chalcone route. This route attracts the attention because of its simple operating conditions and easy availability of the chemicals. Initially, benzylideneacetophenones (chalcones) were prepared in high yields by Claisen-Schmidt reaction of acetophenones with 4-(methylthio)benzaldehyde. The cyclopropyl ketones were prepared in good yields by the reaction of chalcones with trimethylsulfoxonium iodide. Tetralones were prepared in good yields by the Friedel-Craft's intramolecular cyclization reaction of cyclopropyle ketones in the presence of anhyd. stannic chloride and acetic anhydride. The tetralones on formylation to give substituted hydroxylmethylene tetralones. Condensation of substituted hydroxylmethylene tetralones with hydrazine hydrate afforded target compounds. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral technique. The title compounds were screened for their antimitotic and antimicrobial activities. Among the synthesized compounds cyclopropyl ketones and pyrazole analogues of podophyllotoxin, compound 7-(methylthio)-5-(4-(methylthio)phenyl)-4,5-dihydro-^{2}*H*-benzo[g]indazole is more active than 5-(4-(methylthio)phenyl)-4,5-dihydro-^{2}*H*-benzo[g]indazole, 7-methyl-5-(4-(methylthio)phenyl)-4,5-dihydro-^{2}*H*-benzo[g]indazole, 7-methoxy-5-(4-(methylthio)phenyl)-4,5-dihydro-^{2}*H*-benzo[g]indazole and the key intermediate tetralones in 100, 200 and 400 ppm at 12, 18 and 24 h and also showed very good activity against screened bacteria and fungi compared to their standard.

Keywords: acetophenones, chalcones, cyclopropylketones, tetralones, antimitotic activity, antimicrobial activity

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INTRODUCTION

Podophyllotoxin (Fig. 1) is a most abundant naturally occurring antimitotic cyclolignan, derived from variety of plants, such as podophyllum peltalum, podophyllum emodi, podophyllum pleianthum, podophyllum hexandrum anthriscus sylvertris and juniperus Sabina [1–7]. Podophyllotoxin exhibits high antimitotic and apoptotic activity, this is due to its affinity for tubulin and mitotic spindles of dividing cells at metaphase. Since it is having high toxicity, it reduces the application of podophyllotoxin as an anticancer agent. The toxicity of podophyllotoxin liberates as diarrhea, nausea, vomiting and damages to the healthy tissues [8, 9]. At the same time heterocyclic ring systems have emerged as powerful scaffolds for many biological evaluations [10]. Heterocyclic compounds provide scaffolds on which pharmacophores can arrange to yield potent and selective drugs [11]. Pyrazoles and their derivatives play an important role in medicinal chemistry research. Several derivatives of pyrazole are of pharmaceutical interest due to their analgesic power. Among them, derivatives of 5-isopyrazolone and pyrazolidine-3,5-dione are worth noting due to their clinical interest. Several, substituted pyrazolines are reported to possess moderate antibacterial and antifungal activities. Besides, a number of nitrofurylpyrazoline derivatives were found to possess antibacterial activity. Pyrazole molecules are also exhibit anticancer, anti-inflammatory, antidepressant, anticonvulsant [12]. The incorporation of pyrazole ring into the podophyllotoxin enhances the biological activities to a great extent and also presence of differ-

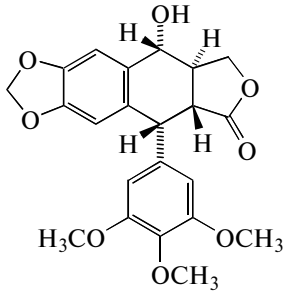

Fig. 1. Podophyllotoxin.

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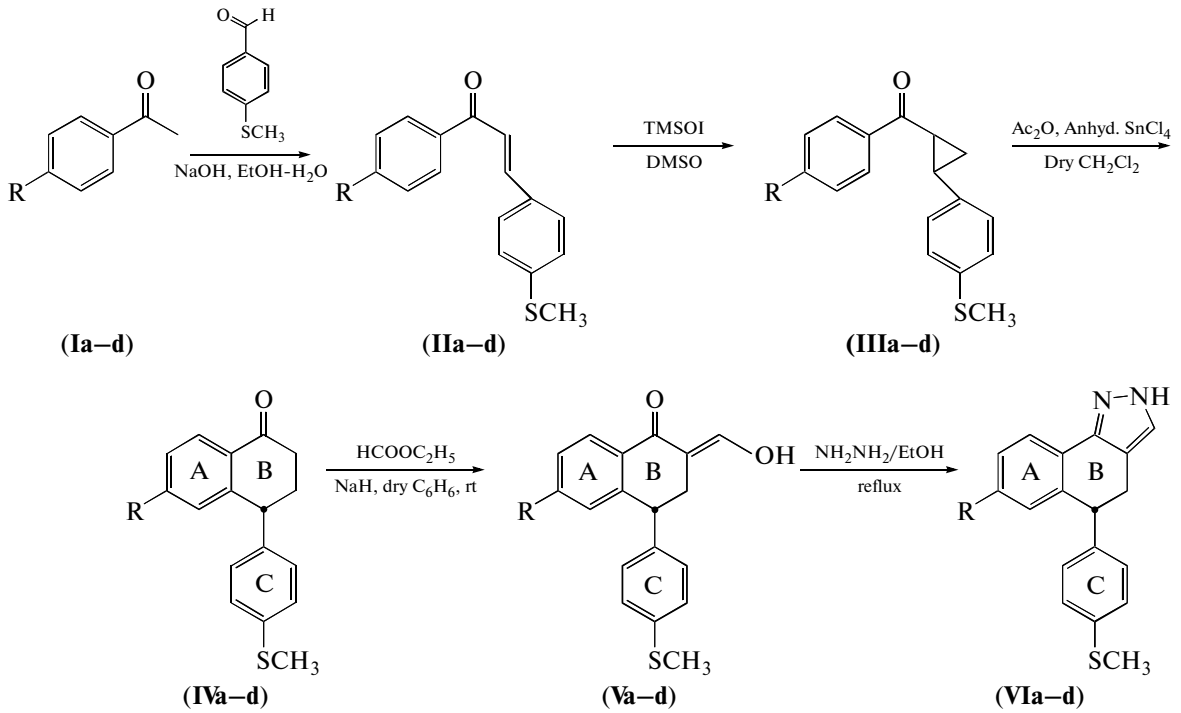
ent substituents on the new pyrazole analogues of podophyllotoxin, well improve the biological properties. Hence these modifications of its structure are required to reduce its toxicity and to enhance its biological activity. The biologically active and less cytotoxic new pyrazole analogues of podophyllotoxin have been synthesized.

RESULTS AND DISCUSSION

The synthesis of new pyrazole analogues of podophyllotoxin has been carried out by chalcone route (Scheme 1). The benzylideneacetophenones (chalcones) (**IIa**–**d**) were prepared in high yields by Claisen-Schmidt reaction of acetophenones (**Ia**–**d**) with 4-(methylthio)benzaldehyde in the presence of sodium hydroxide in water-ethanol mixture [13, 14]. The structures of the chalcones were confirmed by IR and ^{1}H NMR spectral studies. IR spectra of compounds (**IIa**–**d**) showed the C=C stretching frequency in the range 1667–1656 cm^{-1} and ^{1}H NMR showed the absence of aldehyde proton at 9.83 ppm. The cyclopropyl ketones (**IIIa**–**d**) were prepared in good yields by the reaction of chalcones (**IIa**–**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 ketone. The structure of compounds (**IIIa**–**d**) was confirmed by IR spectra. In its IR spectra exhibit 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 0.83–0.78 and 2.21–2.00 ppm respectively. Tetralones (**IVa**–**d**) were prepared in good yields by the Friedel-Craft's intramolecular cyclization reaction of cyclopropyle ketones (**IIIa**–**d**) in the presence of anhyd. stannic chloride and acetic anhydride in dry dichloromethane [17]. The cyclopropyl ketones undergo 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 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 to aromatic 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 preparation of the new pyrazole linked podophyllotoxin analogues.



(**Ia**)–(**VIa**) R = H; (**Ib**)–(**VIb**) R = CH_3; (**Ic**)–(**VIc**) R = OCH_3; (**Id**)–(**VId**) R = SCH_3

Scheme 1. Protocol for synthesis of tetralones (**IVa**–**d**).

The tetralones on formylation to give substituted hydroxylmethylene tetralones (**Va–d**) [18]. Formylation of the presently synthesized tetralones with ethyl formate using sodium hydride as the base at room temperature gave single product in good yields. The β-dicarbonyl compounds which exist in the enol form show the carbonyl absorption in the region 1640–1610 cm^{-1}. Generally 1,3-diketones absorption peak with high intensity appeared at 1715 cm^{-1}. Enols containing this grouping, but the shift that occurs in these compounds is attributed to the intramolecular hydrogen bonding.

At the same time, the true alcoholic hydroxyl band near 3530 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. ^1H NMR showed the vinylic proton absorption at 5.80–5.60 ppm. The new pyrazole analogues of podophyllotoxin (**VIa–d**) were synthesized in high yields by the condensation of hydroxymethylene tetralones and hydrazine hydrate in absolute ethanol [19]. The products were purified by recrystallization from ethanol. The compounds (**VIa–d**) exhibited NH stretching band at 3490–3360 cm^{-1} and proton NMR showed singlet NH peak at 12.64–12.55 ppm. Based on this, the structures of the synthesized compounds were confirmed.

Biological Activities

Antimitotic Activity

Allium cepa has been used to evaluate the antimitotic activity of new pyrazole analogues of podophyllotoxin. Root tip cells in (**IVa–d**) and (**VIa–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 compound (**IVa–d**) and (**VIa–d**) of 100, 200 and 400 ppm at 12, 18 and 24 h exhibited changes in chromosomes and shape of the cells with elongated appearance. Using cytotoxic nature of new pyrazole 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 compounds (**IVa–d**) and (**VIa–d**) exhibited less change in cell shape with elongated appearance. (**IVa–d**) and (**VIa–d**) compounds of 100, 200 and 400 ppm were used for this experiment, pyrazole moiety present at ring B of (**VIa–d**) compounds showed highest antimitotic activity when compared to (**IVa–d**) compounds in 100, 200 and 400 ppm at 12, 18 and 24 h. Among (**VIa–d**), compound **VId** possessing *thiomethyl* substituent on ring A and C showed lowest mitotic index there by indicating highest level of antimitotic activity in 400 ppm concentration at 24 h. Where as compounds **VIb** and **VIc** having *methyl* and *methoxy* group present on ring A respectively and *thiomethyl* substituent on ring C showed moderate activity and compound **VIa** having *thiomethyl* group exhibited highest mitotic index with lowest ability to inhibit the cell growth in 100, 200 and 400 ppm at 12, 18 and 24 h. It can be said that compound **VId** has maximum inhibitory effect compared to key intermediate (**IVa–d**) and compounds **VIa, VIb** and **VIc** in 100, 200 and 400 ppm at 12, 18 and 24 h exhibited inhibitory effect on the cell division of the onion root.

From the 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 12, 18 and 24 h time duration (Figs. 2a–2f). Therefore antimitotic ability of new pyrazole analogues of podophyllotoxin was remarkable in controlling the cell division and hence acts as a very good antimitotic agent. The results of antimitotic activity are given in (Tables 1 and 2).

Antimicrobial Activity

The antibacterial screening revealed that some of the compounds showed good inhibition against various tested microbial strains. The key intermediate compounds (**IVa–d**) possessed moderate antibacterial and antifungal activities (Table 3). Further, hydrazine was condensed with (**Va–d**) gave new pyrazole analogues of podophyllotoxin accounted for the enhanced activities (Table 4). The results indicated that **VIa** having *thiomethyl* substituent present at ring C exhibited least activity and **VIb** and **VIc** possessing *thiomethyl* substituent present at ring C and *methyl* and *methoxy* group on ring A showed considerable activity and at the same time **VId** possessing *thiomethyl* substituent present at ring A and C showed good activity against all screened bacteria compared to **VIa**, **VIb**, **Vic** and key intermediate compounds (**IVa–d**) at concentration of 10.0 mg/mL compared to standard drug Chloramphenicol.

Newly synthesized compounds (**IVa–d**) and (**VIa–d**) were also screened for their antifungal activity against two tested microbial strains (Tables 5 and 6). Among the tested compounds, compound **VIa** exhibited least activity and **VIb** and **VIc** showed moderate activity where as **VId** showed very good activity against two treated fungi compared to **VIa, VIb, VIc** and key intermediate compounds (**IVa–d**) at concentration of 10.0 mg/mL compared to standard drug Nystatin.

In summary, new pyrazole analogues of podophyllotoxin were synthesized in good yields by chalcone route. The synthesized compounds were screened for their antimitotic and antimicrobial activity. Among the synthesized compounds (**IVa–d**) and (**VIa–d**), compound **VId** is more active than **VIa, VIb, VIc** and the key intermediates (**IVa–d**) by displaying the antimitotic and antimicrobial activity at 10.0 mg/L concentration. The compounds (**VIa–d**) showed better antimitotic and antimicrobial activity than the compounds (**IVa–d**) in 100, 200, and 400 ppm at 12, 18 and 24 h.

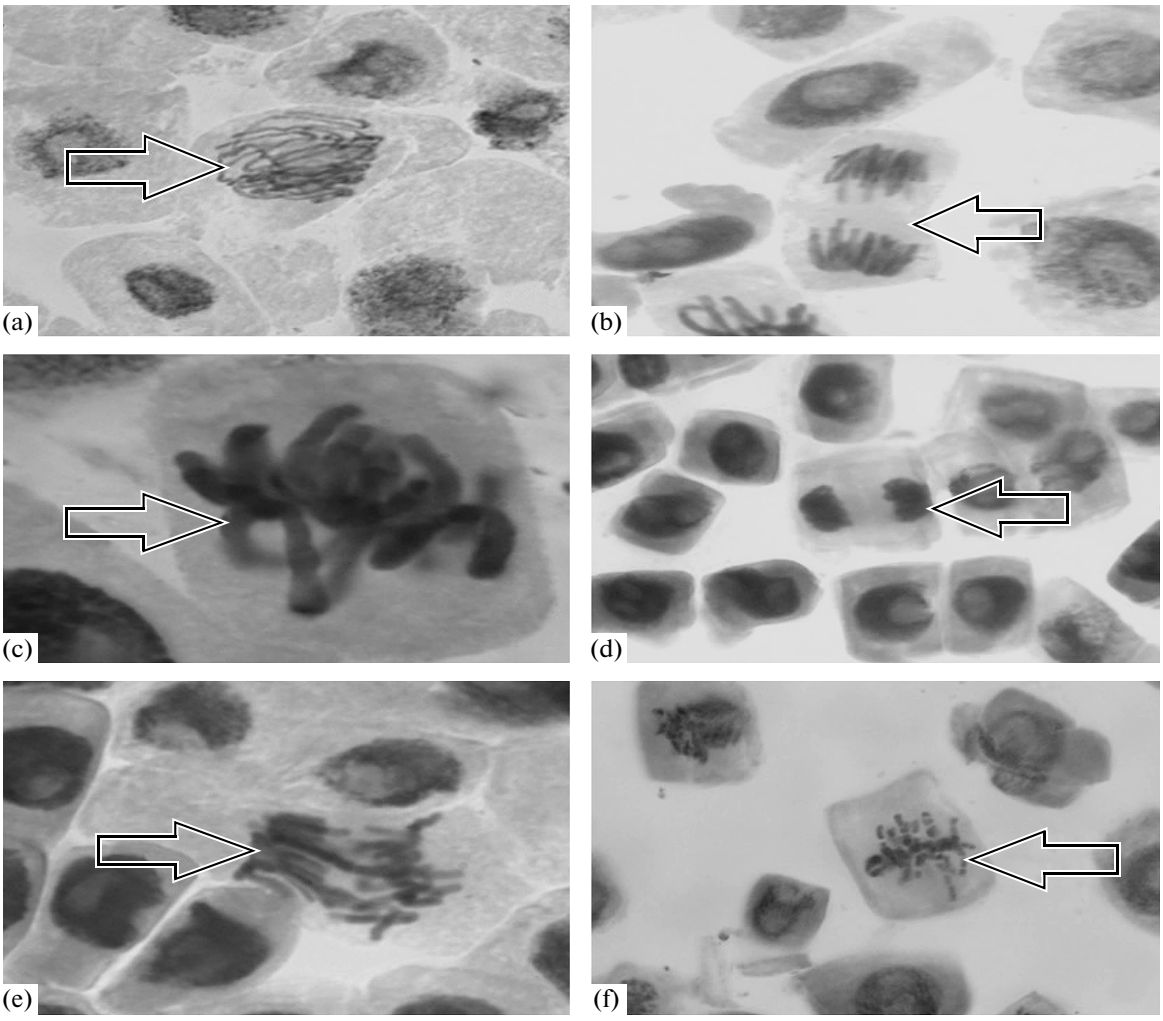


Fig. 2. (a) Prophase, (b) anaphase, (c) metaphase, (d) telophase, (e) chromosomal bridge, and (f) chromosomal breakage.

EXPERIMENTAL

All reagents and chemicals were purchased from Merck Chemicals used without further purification. Melting points were taken in open capillary tubes and are uncorrected. 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 (ν, cm^{-1}) were recorded on Perkin-Elmer model 683 spectrometers.; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (δ, ppm; *J*, Hz) using trimethyl silane (TMS) as an internal reference were recorded in $CDCl_3$ on Bruker spectrometer, Elemental analyses were performed on a Perkin-Elmer 2400. Mass spectra ESI were obtained by Water-Q-TOF ultima spectrometer. Micro analytical data were obtained by elemental-Vario EL-III.

General procedure for the synthesis of chalcones (IIa–d). Substituted acetophenones (0.05 mol) (**Ia–d**) and 4-(methylthio) benzaldehyde (6.64 g, 0.05 mol) were stirred in water (40 mL) and ethanol (25 mL) mixture in the presence of sodium hydroxide (2.00 g, 0.05 mol) at 15–30°C for 4 h. The reaction mixture was kept overnight in an ice bath. The precipitated products were filtered and recrystallized from ethanol.

General procedure for the synthesis of cyclopropyl ketones analogues (IIIa–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 DMSO (20 mL) under nitrogen gas atmosphere. The reaction mixture was stirred for 10 min at 25–30°C (until the evolution of the H_2 gas ceased). Chalcones (0.02 mol) (**IIa–d**) in dry DMSO (15 mL) were added drop wise during 30 min to the above solution. The reaction mass was stirred at 26–28°C for 2 h and raised the temperature to 50–60°C for 1 h. The completion of the reaction was confirmed by TLC and 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

Table 1. Antimitotic activity of the compounds (**IVa–d**) by onion root tip method

Comp. No.	Conc. in ppm	% Dividing cells			% Dividing cells compared to control			% Inhibition compared to control			ID_{50} in ppm		
		time, h											
		12	18	24	12	18	24	12	18	24	12	18	24
Control	–	32.91	24.72	21.74	100			0.0			–		
(IVa)	100	22.24	20.03	14.50	67.57	81.02	66.69	32.43	18.98	33.31			
	200	19.91	17.02	13.23	60.49	68.85	60.35	39.51	31.15	39.15	380	390	400
	400	15.85	11.99	10.77	48.16	48.50	49.54	51.84	51.50	50.46			
(IVb)	100	19.02	17.18	17.01	57.79	69.49	78.24	42.21	30.51	21.76			
	200	17.47	12.01	13.13	53.08	48.58	60.39	46.92	51.42	39.61	320	190	370
	400	15.51	10.00	10.56	47.12	40.45	48.57	52.88	59.55	51.43			
(IVc)	100	19.99	18.48	15.45	60.74	74.75	71.06	39.26	25.25	28.94			
	200	16.66	15.56	13.00	50.63	62.94	59.79	49.37	37.06	40.21	220	350	350
	300	14.56	11.45	10.01	44.24	46.31	46.04	55.76	53.69	53.96			
(IVd)	100	17.56	13.01	13.20	53.35	52.62	60.71	46.65	47.38	39.29			
	200	15.69	09.56	09.21	45.85	39.88	42.36	54.15	60.12	57.64	140	120	160
	400	11.78	07.09	05.45	35.79	28.68	25.06	64.21	71.32	74.94			

Table 2. Antimitotic activity of the compounds (**VIa–d**) by onion root tip method

Comp. No.	Conc. in ppm	% Dividing cells			% Dividing cells compared to control			% Inhibition compared to control			ID_{50} in ppm		
		time, h											
		12	18	24	12	18	24	12	18	24	12	18	24
Control	–	41.16	35.04	31.32	100			0.0			–		
(VIa)	100	25.43	28.33	20.34	61.78	80.86	64.94	38.22	19.14	35.05			
	200	23.61	16.81	18.09	57.36	47.97	57.75	42.64	52.02	42.25	330	190	400
	400	19.03	14.56	15.58	46.23	41.55	49.74	53.77	58.44	50.26			
(VIb)	100	22.90	21.66	19.87	55.63	61.81	63.44	44.37	38.18	36.56			
	200	20.01	15.72	21.10	48.61	44.86	67.36	51.38	55.13	32.64	180	170	370
	400	17.81	13.32	14.74	43.27	38.01	47.06	56.72	61.98	52.94			
(VIc)	100	23.41	22.23	22.52	56.87	63.44	71.90	43.12	36.55	28.10			
	200	20.01	18.41	14.90	48.61	52.53	47.57	51.38	47.46	52.43	180	240	190
	300	16.66	14.03	12.83	40.47	40.03	40.96	59.52	59.96	59.04			
(VId)	100	22.70	21.41	19.29	55.15	61.11	61.59	44.84	38.89	38.41			
	200	19.68	12.24	13.32	47.81	34.93	42.52	52.18	65.07	57.48	170	150	160
	400	15.57	07.92	06.27	37.82	22.60	20.01	62.17	77.40	79.99			

and concentrated under reduced pressure. The products were recrystallized from ethanol.

General procedure for the synthesis of key intermediate tetralones (IVa–d). Cyclopropyl ketones (0.01 mol) (**IIIa–d**) were 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°C for 3 h. 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)

Table 3. Antibacterial activity of the compounds (**IVa–d**). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Comp. No.	*E. faecalis*	*K. pneumonia*	*E. aerogenes*	*P. aeruginosa*	*A. faecalis*
Conc. in mg/mL	10.0	10.0	10.0	10.0	10.0
Control	00	00	00	00	00
Chloramphenicol	18.4 ± 0.08	18.8 ± 0.06	19.2 ± 0.04	18.6 ± 0.06	18.4 ± 0.03
(IVa)	14.2 ± 0.02	12.2 ± 0.04	11.1 ± 0.06	12.4 ± 0.02	12.1 ± 0.08
(IVb)	12.4 ± 0.03	14.1 ± 0.06	12.2 ± 0.06	14.1 ± 0.04	13.1 ± 0.02
(IVc)	15.6 ± 0.04	14.1 ± 0.02	12.8 ± 0.02	12.9 ± 0.02	13.1 ± 0.02
(IVd)	16.0 ± 0.03	15.8 ± 0.04	13.2 ± 0.0.2	13.8 ± 0.04	14.3 ± 0.04

Values are means of triplicates.
Standard 10 mg/disc.

Table 4. Antibacterial activity of the compounds (**VIa–d**). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Comp. No.	*E. faecalis*	*K. pneumonia*	*E. aerogenes*	*P. aeruginosa*	*A. faecalis*
Conc. in mg/mL	10.0	10.0	10.0	10.0	10.0
Control	00	00	00	00	00
Chloramphenicol	18.4 ± 0.08	18.8 ± 0.06	19.2 ± 0.04	18.6 ± 0.06	18.4 ± 0.03
(VIa)	16.4 ± 0.02	15.2 ± 0.04	14.1 ± 0.02	15.4 ± 0.04	14.8 ± 0.02
(VIb)	13.4 ± 0.02	15.8 ± 0.03	13.5 ± 0.03	15.6 ± 0.02	14.4 ± 0.04
(VIc)	16.4 ± 0.04	15.9 ± 0.02	14.1 ± 0.04	14.6 ± 0.03	15.1 ± 0.02
(VId)	17.2 ± 0.02	18.0 ± 0.04	16.2 ± 0.02	16.9 ± 0.04	17.4 ± 0.04

Values are Mean of triplicates.
Standard 10 mg/disc.

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.

Table 5. Antifungal activity of the compounds (**IVa–d**). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Comp. No.	*F. verticillioides*	*A. niger*
Conc. in mg/mL	10.0	10.0
Control	00	00
Nystatin	22.0 ± 0.04	18.0 ± 0.06
(IVa)	12.4 ± 0.02	08.2 ± 0.02
(IVb)	08.1 ± 0.04	06.4 ± 0.04
(IVc)	13.6 ± 0.04	09.8 ± 0.04
(IVd)	16.1 ± 0.02	15.2 ± 0.02

Values are Mean of triplicates.
Standard 10 mg/disc.

General procedure for the synthesis of substituted hydroxyl methylene tetralones (Va–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 h. Ethyle formate (10 mL) was added dropwise to the above reaction mixture and stirred for another 1 h, followed by dropwise addition of tetralones (0.05 mol) (**IVa–d**), in dry benzene (100 mL) over a period of 1 h. After stirring the red coloured mixture at room temperature for 2 h, it was poured into 2 N H_2SO_4 (100 mL) in ice. The separated organic layer was washed with water (3 × 50 mL) and extracted into 1% sodium hydroxide solution (3 × 50 mL). The sodium hydroxide extract was acidified with 2 N H_2SO_4 gave products in good yields. They were recrystallized from ethanol.

General procedure for the synthesis of new pyrazole analogues of podophyllotoxin (VIa–d). A mixture of hydroxymethylene tetralones (0.01 mol) (**Va–d**) and hydrazine (0.49 mL, 0.01 mol) in absolute ethanol was refluxed for 3 h. The excess of solvent was removed

under reduced pressure. The solid thus obtained were collected and recrystallized from ethanol.

3-(4-(Methylthio)phenyl)-1-phenylprop-2-en-1-one (IIa). Color: light yellow solid. Yield: 85.90%. mp: 95–97°C. IR: 3150–2972 (Ar-CH), 1677 (C=O), 1543 (C=C); ^{1}H NMR: 8.05 (d, 1H, J = 7.8, β-CH), 7.80–7.28 (9 H, m, Ar-H), 7.62 (1 H, d, J = 7.8, α-CH), 2.52 (3 H, s, SCH_3); ^{13}C NMR: 189.6, 145.3, 139.5, 137.9, 135.6, 134.6, 130.8, 129.3, 128.7, 125.8, 124.9, 124.8, 121.5, 14.9; MS, m/z: 254.10 (M^+). Anal. calcd. for $C_{17}H_{14}OS$: C, 86.40; H, 6.82. Found: C, 86.32; H, 6.78%.

3-(4-(Methylthio)phenyl)-1-*p*-tolylprop-2-en-1-one (IIb). Color: light yellow solid. Yield: 79.25%. mp: 111–113°C. IR: 3158–2968 (Ar-CH), 1683 (C=O), 1558 (C=C); ^{1}H NMR: 8.09 (1 H, d, J = 7.5, β-CH), 7.85–7.32 (8 H, m, Ar-H), 7.58 (1 H, d, J = 7.3, α-CH), 2.54 (3 H, s, SCH_3), 2.32 (3 H, s, CH_3); ^{13}C NMR: 189.5, 145.5, 144.5, 139.5, 135.6, 134.9, 130.8, 129.9, 129.6, 125.3, 124.9, 124.7, 121.5, 21.6, 14.3; MS, m/z: 268.15 (M^+). Anal. calcd. for $C_{17}H_{16}OS$: C, 76.08; H, 6.01. Found: C, 76.05; H, 6.03%.

1-(4-Methoxyphenyl)-3-(4-(methylthio)phenyl)prop-2-en-1-one (IIc). Color: light yellow solid. Yield: 80.10%. mp: 98–100°C. IR: 3162–2953 (Ar-CH), 1675 (C=O), 1549 (C=C); ^{1}H NMR: 7.98 (1 H, d, J = 8.2, β-CH), 7.91–7.32 (8 H, m, Ar-H), 7.61 (1 H, d, J = 8.2, α-CH), 3.82 (3 H, s, OCH_3), 2.56 (3 H, s, SCH_3); ^{13}C NMR: 189.4, 166.5, 145.3, 139.5, 135.6, 130.9, 130.8, 130.5, 130.3, 125.9, 124.9, 124.7, 121.5, 114.9, 55.5, 14.3; MS, m/z: 284.14 (M^+). Anal. calcd. for $C_{17}H_{16}O_2S$: C, 71.80; H, 5.67. Found: C, 71.83; H, 5.66%.

1,3-bis(4-(Methylthio)phenyl)prop-2-en-1-one (IId). Color: light yellow solid. Yield: 76.03%. mp: 120–122°C. IR (KBr, ν, cm^{-1}): 3158–2962 (Ar-CH), 1680 (C=O), 1553 (C=C); ^{1}H NMR: 8.02 (1 H, d, J = 8.2, β-CH), 7.85–7.35 (8 H, m, Ar-H), 7.58 (1 H, d, J = 8.2, α-CH), 2.54 (6 H, s, SCH_3); ^{13}C NMR: 189.3, 145.6, 145.2, 139.5, 135.6, 135.4, 134.4, 130.7, 128.9, 127.5, 125.9, 124.9, 124.7, 121.5, 14.5; MS, m/z: 300.15 (M^+). Anal. calcd. for $C_{17}H_{16}OS_2$: C, 67.96; H, 5.37. found: C, 67.95; H, 5.39%.

(2-(4-(Methylthio)phenyl)cyclopropyl)(phenyl)methanone (IIIa). Color: dark brown semisolid. Yield: 71.40%. IR: 3183–2972 (Ar-CH), 1685 (C=O), 1273 (C–C); ^{1}H NMR: 7.90–7.32 (9 H, m, Ar-H), 2.58 (3 H, s, SCH_3), 2.16–2.00 (2 H, m, cyclopro-CH), 0.80 (2 H, d, J = 6.8, cyclopro-CH_2); ^{13}C NMR: 192.3, 143.6, 138.1, 136.5, 133.2, 128.9, 126.6, 128.1, 125.9, 121.5, 120.3, 27.1, 24.6, 14.6, 14.5; MS, m/z: 268.12 (M^+). Anal. calcd. for $C_{17}H_{16}OS$: C, 76.08; H, 6.01. Found: C, 76.07; H, 6.03%.

(2-(4-(Methylthio)phenyl)cyclopropyl)(*p*-tolyl)methanone (IIIb). Color: dark brown semisolid. Yield: 69.65%. IR: 3186–2974 (Ar-CH), 1688 (C=O), 1270 (C–C); ^{1}H NMR: 7.97–7.35 (8 H, m, Ar-H), 2.58 (3 H, s, SCH_3), 2.32 (3 H, s, CH_3), 2.18–2.02 (2 H, m, cyclopro-CH), 0.81 (2 H, d, J = 6.6, cyclopro-CH_2); ^{13}C NMR: 192.5, 143.1, 142.5, 138.1, 133.3, 128.7, 128.5, 128.1. 125.9. 121.5, 120.4, 27.6, 24.8, 21.1, 14.8, 14.5; MS, m/z: 282.10 (M^+). Anal. calcd. for $C_{18}H_{18}OS$: C, 76.56; H, 6.42. Found: C, 76.55; H, 6.44%.

Table 6. Antifungal activity of the compounds (**VIa–d**). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Comp. No.	*F. verticillioides*	*A. niger*
Conc. in mg/mL	10.0	10.0
Control	00	00
Nystatin	22.0 ± 0.04	18.0 ± 0.06
(VIa)	13.6 ± 0.04	09.8 ± 0.04
(VIb)	10.1 ± 0.02	09.2 ± 0.02
(VIc)	16.6 ± 0.02	14.2 ± 0.04
(VId)	20.8 ± 0.02	17.6 ± 0.04

Values are means of triplicates.
Standard 10 mg/disc.

(4-Methoxyphenyl)(2-(4-(methylthio)phenyl)cyclopropyl)methanone (IIIc). Color: dark brown semisolid. Yield: 76.90%. IR: 3183–2975 (Ar-CH), 1689 (C=O), 1269 (C–C); ^{1}H NMR: 7.98–7.31 (8 H, m, Ar-H), 3.82 (3 H, s, OCH_3), 2.57 (3 H, s, SCH_3), 2.15–2.01 (2 H, m, cyclopro-CH), 0.84 (2 H, d, J = 6.3, cyclopro-CH_2); ^{13}C NMR: 192.1, 165.1, 143.6, 138.3, 129.9, 129.0, 128.2, 125.9, 121.4, 120.4, 114.5, 55.5, 27.4, 24.9, 14.6, 14.5; MS, m/z: 298.13 (M^+). Anal. calcd. for $C_{18}H_{18}O_2S$: C, 72.45; H, 6.08. Found: C, 72.42; H, 6.07%.

(4-(Methylthio)phenyl)-(2-(4-(methylthio)phenyl)cyclopropyl)methanone (IIId). Color: dark brown semisolid. Yield: 65.35%. IR: 3181–2976 (Ar-CH), 1691 (C=O), 1273 (C-C); ^{1}H NMR: 7.96–7.33 (8 H, m, Ar-H), 2.55 (6 H, s, SCH_3), 2.11–2.00 (2 H, m, cyclopro-CH), 0.87 (2 H, d, J = 6.8, cyclopro-CH_2); ^{13}C NMR: 192.3, 143.6, 143.5, 138.4, 133.3, 132.0, 128.5, 126.6, 125.4, 121.3, 120.3, 27.4, 24.8, 14.9, 14.4; MS, m/z: 314.13 (M^+). Anal. calcd. for $C_{18}H_{18}OS_2$: C, 68.75; H, 5.77. Found: C, 68.72; H, 5.78%.

4-(4-(Methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (IVa). Color: dark brown gummy solid. Yield: 78.81%. IR: 3128–2935 (Ar-CH), 1691 (C=O); ^{1}H NMR : 7.83–7.33 (9 H, m, Ar-H), 4.22 (1 H, t, J = 4.6, CH), 2.64–2.26 (4 H, tt, J = 6.3, CH_2), 2.55 (3 H, s, SCH_3), ^{13}C NMR: 198.3, 141.6, 140.9, 140.5, 134.7, 133.6, 129.5, 128.5, 128.2, 126.5, 45.5, 37.6, 31.4, 14.9; MS, m/z: 268.07 (M^+). Anal.

calcd. for $C_{17}H_{16}OS$: C, 76.08; H, 6.01. Found: C, 76.09; H, 6.03%.

6-Methyl-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (VIb). Color: dark brown gummy solid. Yield: 71.94%. IR: 3125–2938 (Ar-CH), 1695 (C=O); ^{1}H NMR: 7.89–7.32 (7 H, m, Ar-H), 4.26 (1 H, t, J = 4.8, CH), 2.65–2.28 (4 H, tt, J = 6.5, CH_2), 2.54 (3 H, s, SCH_3), 2.34 (3 H, s, CH_3); ^{13}C NMR: 198.1, 143.6, 141.4, 140.6, 140.1, 131.0, 129.6, 128.5, 128.1, 126.1, 125.4, 45.9, 37.6, 31.6, 21.5, 14.5; MS, *m/z*: 282.14 (M^+). Anal. calcd. for $C_{18}H_{18}OS$: C, 76.56; H, 6.42. Found: C, 76.58; H, 6.45%.

6-Methoxy-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (IVc). Color: dark brown gummy solid. Yield: 75.18%. IR: 3128–2939 (Ar-CH), 1697 (C=O); ^{1}H NMR: 7.88–7.35 (7 H, m, Ar-H), 4.24 (1 H, t, J = 4.7, CH), 2.66–2.25 (4 H, tt, J = 6.4, CH_2), 3.84 (3 H, s, OCH_3), 2.53 (3 H, s, SCH_3); ^{13}C NMR: 198.0, 165.9, 141.7, 140.1, 130.5, 129.5, 128.5, 126.5, 111.9, 104.7, 55.7, 45.6, 37.4, 31.4, 14.5; MS, *m/z*: 298.15 (M^+). Anal. calcd. for $C_{18}H_{18}O_2S$: C, 72.45; H, 6.08. Found: C, 72.40; H, 6.09%.

6-(Methylthio)-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (IVd). Color: dark brown gummy solid. Yield: 64.71%. IR (KBr, ν, cm^{-1}): 3125–2938 (Ar-CH), 1688 (C=O); ^{1}H NMR: 7.84–7.21 (7 H, m, Ar-H), 4.21 (1 H, t, J= 4.6, CH), 2.61–2.10 (4 H, tt, J= 5.9, CH_2), 2.56 (6 H, s, SCH_3); ^{13}C NMR: 198.2, 1439, 141.5, 140.6, 140.0, 130.5, 129.4, 129.3, 128.6, 124.4, 123.2, 44.7, 37.4, 31.3, 14.8; MS, *m/z*: 314.12 (M^+). Anal. calcd. for $C_{18}H_{18}OS_2$: C, 68.75; H, 5.77. Found: C, 68.73; H, 5.74%.

2-(Hydroxymethylene)-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (Va). Color: dark brown solid. Yield: 81.63%. mp: 151–153°C. IR (KBr, ν, cm^{-1}): 3268 (O–H), 3170–2964 (Ar-CH), 1695 (C=O); ^{1}H NMR ($CDCl_3$ 400 MHz) δ ppm: 7.74–7.17 (8 H, m, Ar-H), 5.8 (1 H, bs, OH vinyl), 5.37 (1 H, s, CHOH), 4.05 (1 H, t, J = 5.4, CH), 2.76 (2 H, d, J = 6.1, CH_2), 2.54 (3 H, s, SCH_3); ^{13}C NMR ($CDCl_3$ 100 MHz) δ ppm: 183.4, 172.6, 141.6, 140.3, 139.5, 135.2, 133.4, 129.3, 128.7, 128.6, 127.9, 126.4, 117.3, 42.5, 35.5, 14.6; MS, *m/z*: 296.11 (M^+). Anal. calcd. for $C_{18}H_{16}O_2S$: C, 72.94; H, 5.44. Found: C, 72.93; H, 5.42%.

2-(Hydroxymethylene)-6-methyl-4-(4-(methylthio)-phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (Vb). Color: dark brown solid. Yield: 84.48%. mp: 149–151°C. IR: 3269 (O–H), 3177–2968 (Ar-CH), 1699 (C=O); ^{1}H NMR: 7.73–7.15 (7 H, m, Ar-H), 5.8 (1 H, bs, OH vinyl), 5.38 (1 H, s, CHOH), 4.06 (1 H, t, J = 5.6, CH), 2.84 (3 H, s, CH_3), 2.77 (2 H, d, J = 6.3, CH_2), 2.55 (3 H, s, SCH_3); ^{13}C NMR: 183.6, 172.4, 144.9, 141.2, 140.6, 139.5, 130.5, 130.0, 129.5, 128.5, 128.4, 127.2, 117.2, 42.8, 35.3, 21.8, 14.6 ; MS, *m/z*: 310.12 (M^+). Anal. calcd. for $C_{19}H_{18}O_2S$: C, 73.52; H, 5.84. Found: C, 73.50; H, 5.85%.

2-(Hydroxymethylene)-6-methoxy-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (Vc). Color: dark brown solid. Yield: 85.95%. mp: 162–164°C. IR: 3267 (O–H), 3175–2969 (Ar-CH), 1695 (C=O); ^{1}H NMR: 7.76–7.18 (7 H, m, Ar-H), 5.7 (1 H, bs, OH vinyl), 5.39 (1 H, s, CHOH), 4.07 (1 H, t, J = 5.2, CH), 3.84 (3 H, s, OCH_3), 2.76 (2 H, d, J = 6.4, CH_2), 2.52 (3 H, s, SCH_3); ^{13}C NMR: 183.3, 172.4, 166.8, 142.8, 140.2, 139.5, 129.5, 128.6, 125.1, 117.5, 112.5, 105.3, 55.6, 42.6, 35.5, 14.2; MS, *m/z*: 326.14 (M^+). Anal. calcd. for $C_{19}H_{18}O_3S$: C, 69.91; H, 5.56. Found: C, 69.93; H, 5.55%.

2-(Hydroxymethylene)-6-(methylthio)-4-(4-(methylthio)phenyl)-1,2,3,4-tetrahydronaphthalen-1(2*H*)-one (Vd). Color: dark brown solid. Yield: 61.87%. mp: 175–177°C. IR: 3265 (O–H), 3175–2972 (Ar-CH), 1696 (C=O); ^{1}H NMR: 7.71–7.21 (7 H, m, Ar-H), 5.5 (1 H, bs, OH vinyl), 5.37 (1 H, s, CHOH), 4.09 (1 H, t, J = 5.7, CH), 2.76 (2 H, d, J = 6.3, CH_2), 2.56 (6 H, s, SCH_3); ^{13}C NMR: 183.3, 172.5, 145.3, 141.9, 140.2, 139.6, 130.5, 129.4, 129.1, 128.5, 124.9, 123.6, 117.3, 42.2, 35.1, 14.8; MS (ESI), *m/z*: 342.09 (M^+). Anal. calcd. for $C_{19}H_{18}O_2S_2$: C, 66.63; H, 5.30. Found: C, 66.60; H, 5.33%.

5-(4-(Methylthio)phenyl)-4,5-dihydro-2*H*-benzo[g]indazole (VIa). Color: dark brown solid. Yield: 85.90%. mp: 153–155°C. IR: 3265 (N–H), 3153-2965 (Ar-CH); ^{1}H NMR: 12.48 (1 H, s, NH), 7.69–7.21 (8 H, m, Ar-H), 7.55 (1 H, s, pyrazole-CH), 4.36 (1 H, t, J = 6.7, CH), 3.29 (2 H, dd, J = 4.3, CH_2), 2.54 (3 H, s, SCH_3); ^{13}C NMR: 144.2, 140.9, 140.1, 139.5, 133.4, 129.3, 128.9, 128.6, 126.8, 126.3, 123.5, 120.6, 114.8, 45.9, 37.3, 14.5; MS, *m/z*: 292.13 (M^+). Anal. calcd. for $C_{18}H_{16}N_2S$: C, 73.94; H, 5.52; N, 9.58. Found: C, 73.95; H, 5.50; N, 9.59%.

7-Methyl-5-(4-(methylthio)phenyl)-4,5-dihydro-2*H*-benzo[g]indazole (VIb). Color: dark brown solid. Yield: 75.95%. mp: 148–150°C. IR: 3269 (N–H), 3155–2963 (Ar-CH); ^{1}H NMR: 12.47 (1 H, s, NH), 7.73–7.20 (7 H, m, Ar-H), 7.52 (1 H, s, pyrazole-CH), 4.38 (1 H, t, J = 6.5, CH), 3.28 (2 H, dd, J = 4.1, CH_2), 2.83 (3 H, s, CH_3), 2.52 (s, 3H, SCH_3); ^{13}C NMR: 144.3, 140.5, 140.1, 139.5, 138.9, 133.4, 130.6, 129.4, 128.3, 127.9, 127.3. 125.9, 114.6, 46.2, 37.5, 21.7, 14.9; MS, *m/z*: 306.14 (M^+). Anal. calcd. for $C_{19}H_{18}N_2S$: C, 74.47; H, 5.92; N, 9.14. Found: C, 74.49; H, 5.90; N, 9.15%.

7-Methoxy-5-(4-(methylthio)phenyl)-4,5-dihydro-2*H*-benzo[g]indazole (VIc). Color: dark brown solid. Yield: 81.05%. mp: 163–165°C. IR: 3267 (N–H), 3151–2960 (Ar-CH); ^{1}H NMR: 12.51 (1 H, s, NH), 7.75–7.25 (7 H, m, Ar-H), 7.54 (1 H, s, pyra-

zole-CH), 4.39 (1 H, t, $J = 6.3$, CH), 3.83 (3 H, s, OCH_3), 3.29 (2 H, dd, $J = 4.6$, CH_2), 2.52 (3 H, s, SCH_3); ^{13}C NMR: 161.3, 144.3, 141.7, 140.2, 139.5, 133.2, 129.6, 129.3, 128.6, 121.3, 114.5, 112.9, 112.4, 55.9, 46.0, 37.5; MS, *m/z*: 322.12 (M^+). Anal. calcd. for $C_{19}H_{18}N_2OS$: C, 70.78; H, 5.63; N, 8.69. Found: C, 70.76; H, 5.65; N, 8.67%.

7-(Methylthio)-5-(4-(methylthio)phenyl)-4,5-dihydro-2*H*-benzo[g]indazole (VId). Color: dark brown solid. Yield: 65.91%. mp: 168–170°C. IR: 3269 (N–H), 3155–2963 (Ar-CH); ^1H NMR: 12.55 (1 H, s, NH), 7.76–7.23 (7 H, m, Ar-H), 7.53 (1 H, s, pyrazole-CH), 4.38 (1 H, t, $J = 6.3$, CH), 3.23 (2 H, dd, $J = 4.5$, CH_2), 2.54 (6 H, s, SCH_3); ^{13}C NMR: 144.3, 140.9, 140.3, 139.6, 139.1, 133.4, 129.4, 128.5, 128.4, 125.5, 124.9, 123.9, 114.8, 45.3, 37.2, 14.9; MS, *m/z*: 338.12 (M^+). Anal. calcd. for $C_{19}H_{18}N_2S_2$: C, 67.42; H, 5.36; N, 8.28. Found: C, 67.40; H, 5.39; N, 8.25%.

Testing of Biological Activities

Antimitotic Studies

The antimitotic activity of synthesized new pyrazole analogues of podophyllotoxin (**VIa–d**) were 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 400 ppm). To study the effect of new pyrazole 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 (7 × 3) after removing the old root from it and immersion is continued for 12 h, 18 h and 24 h. 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 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 [20]. 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 was calculated by following method of Fissceja [21]. 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.

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

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 400 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 new pyrazole analogues of podophyllotoxin for antimitotic activity were calculated individually.

Antimicrobial Studies

Antibacterial Activity

The antimicrobial assay was performed by agar disc diffusion method [22, 23]. For antibacterial activity, the molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 μL of the inoculum (1×10^8 Cfu) and poured into the sterile Petri plates (HiMedia). For agar disc diffusion method, the disc (0.7 cm) (HiMedia) 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°C for 24 h. Antibacterial activity of all the new pyrazole 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.

Antifungal Activity

New pyrazole analogues of podophyllotoxin were dissolved in dimethylformamide (DMF) and evaluated for their antifungal activity by disc diffussion 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 (1×10^8 CFU) and poured into the sterile Petri plates (HiMedia) and the disc (0.7 cm) (HiMedia) 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°C for 7 days. Antifungal activity of all the new pyrazole 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.

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