Research Article

Synthesis and Characterisation of New Tetralone Ester Intermediates of Podophyllotoxin Analogues and their Antifungal Activity

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ABSTRACT

Podophyllotoxin, one of the well-known naturally occurring lignan, has been used as the lead compound for the preparation of potent anticancer agents such as etoposide, tenoposide and etopophos. It also exhibits other biological activities. New tetralone ester intermediates of podophyllotoxin analogues were synthesised in very good yields by chalcone route. They were synthesized by introducing respectively with p-tolyl, p-fluorophenyl and cyclohexyl groups on podophyllotoxin was the transformation of the 3, 4, 5-trimethoxy group and also methylthio group was the transformation of the methylenedioxy group to study their structure activity relationship. All the synthesised products were characterized by spectral and elemental analysis data.

Keywords: Thioanisole, Friedel-Crafts acylation reaction, chalcone, ethyl chloroacetate, powdered sodium, cyclopropyl ketoester, tetralone ester.

INTRODUCTION

Podophyllotoxin **1** is naturally occurring lignan compound. It was isolated from the medicinal plants of genus *Podophyllum*[1-4] belongs to the family of Berberidaceae. Podophyllotoxin was found to be highly cytotoxic for its clinical use against human cancers, extensive modifications of 1 have been undertaken which is convereted into two semi-synthetic analogues of podophyllotoxin, namely etoposide (VP-16) and tenoposide (VM-26) are now in clinical use. Podophyllotoxin also showed wide variety of biological activities such as cathartic, cytotoxic antimitotic, anticancer, antiAIDS, antitropical skin disease, antimalarial, virucidal, and fungicidal[5-8].

Podophyllotoxin **1** is found to exhibit strong antimitotic activity, its wider use as the therapeutic agent in the treatment of neoplastic disease is restricted due to toxic side effects, unfavourable solubility properties and its ready epimerization to Picropodophyllin **2** which is not so active. β-apopicropodophyllin **3**, a dehydrated isomerised product of Podophyllotoxin acts as a much stronger antimitotic agent[9]. Many analogues of podophyllotoxin have been synthesized some analogues showed better antimitotic activity than the parent compound podophyllotoxin. With a view to study their structure activity relationship[10], it was decided to synthesise analogues **4, 5** and **6** by replacing methylenedioxy ring with methylthio groups and changing 3,4,5-trimethoxy Phenyl ring C respectively with p-tolyl, p- flourophenyl and cyclohexyl group in **1** and **2**. Several synthetic routes have been reported for the synthesis of Podophyllotoxin and β-apopicropodophyllin. The chalcone route has been chosen to synthesise new tetralone intermediates of podophyllotoxin analogues[11-12].

MATERIALS AND METHODS

Melting points were taken in open capillaries in a heavy paraffin liquid bath and are uncorrected.IR spectra were recorded in KBr and nujol mull on a FTIR, ^{1}H NMR spectra were taken in $CDCl_3$ on a varian T-300 using TMS as an internal standard (chemical shift in δ, PPm) and the mass spectra on Hitachi RMU-61 spectrophotometer. The purity of the compounds were checked by TLC on silica gel plates using benzene and ethyl acetate mixture in 7:0.5 ml ratio's as developing solvents (eluent). The compounds were purified by column chromatography using silica gel (60-120mesh) as adsorbent and benzene as eluent or repeated recrystallization from ethanol or methanol. The starting material thioanisole was prepared in high yield by refluxing thiophenol with stoichiometric amount of dimethyl sulphate in 10 % aqueous sodium hydroxide solution for 4hr.

Synthetic Procedure

4^1-(methyl thio) acetophenone 11: Thioanisole (10g, 0.0805 moles) in acetic anhydride (50ml) containing fused zinc chloride (10.97g, 0.0805mole) was stirred at room temperature for 12hr. After usual workup, the product was obtained as a yellow crystalline compound in 80% yield. m.p. 78-80^{o}C. IR (KBr):1687(C=O), 1599(C=C) cm^{-1}. ^{1}H NMR ($CDCl_3$):2.56δ(s, 3H, S-CH_3), 2.62(s, 3H, –$COCH_3$), 7.23(d, 2H, J=3Hz, C_3-H, C_5-H), 7.76(d, 2H, C_2-H, C_6-H). Found: C, 65.02; H, 5.99, $C_9H_{10}SO$, required: C, 65.06; H, 6.02 %.

(E)-1-(4-(methylthio)phenyl)-3-p-tolylprop-2-en-1-one 13a: (4^1-(methylthio)-acetophenone **11** (5g, 0.03mole)and benzaldehyde **12a** (3.613g,0.03mole) were stirred vigorously in water (40ml) and ethanol(20ml) mixture in the presence of sodium hydroxide (1.2g, 0.03mole) at 10-30^{o}C for 3hr.The reaction mixture on cooling in an ice

bath, the product was formed. It was filtered and recrystallised from ethanol. The yellow crystalline compound was obtained in 92.13% yield (6.9g), m.p.110-113°C. IR (KBr):1663cm^{-1} (C=O), 1591cm^{-1}(aromatic C=C), ^{1}H NMR($CDCl_3$):2.52(s, 3H, S-CH_3), 7.6-7.98(d, 1H, J=12Hz, C_3-H), 7.19-7.78(m,10H, Ar-H & C_2-H). Found: C, 76.06; H, 6.00; $C_{17}H_{16}SO$ required: C, 76.08; H, 6.01%.

(E)-3-(4-fluorophenyl)-1-(4-(methylthio)phenyl)prop-2-en-1-one 13b: was obtained as red crystalline compound in 79.46% yield(6.5g), m.p.130-132°C. IR (KBr):1662cm^{-1}(C=O), 1593 cm^{-1} (C=C), ^{1}H NMR ($CDCl_3$): 2.52δ(s, 3H, S-CH_3), 8.02(d, 1H, J=12Hz, C_3-H), 7.17-7.79(m, 9H, Ar-H, & C_2-H). $C_{16}H_{13}FOS$; Found: C, 70.55; H, 4.79; required: C, 70.56; H, 4.81%.

(E)-3-cyclohexyl-1-(4-(methylthio)phenyl)prop-2-en-1-one 13c: was obtained as pale yellow crystalline compound in 90.9% yield(7.25g), m.p.159-164°C. IR(KBr):1662cm^{-1} (C=O), 1598cm^{-1}(C=C), ^{1}HNMR($CDCl_3$): 2.52δ(s, 3H, S-CH_3), 6.89-7.05(d,1H,J=12Hz;C_3-H), 7.55-7.78(m, 4H,Ar-H,C_2 -H); 1.32-2.11(m, 11H, cyclo-H) Found: C,73.78; H,7.73; $C_{16}H_{20}OS$, required: C, 73.80; H, 7.74%.

(3R)-ethyl 2-(4-(methylthio)benzoyl)-3-p-tolylcyclopropanecarboxylate 14a: Chalcone **13a** (5g, 0.018 mole), freshly distilled ethyl chloro acetate(2.28g, 0.018mole) and powdered sodium (0.827g, 0.036mole) were stirred in dry benzene (120ml) at room temperature for 30hr.The unreacted sodium and its salts were filtered off. The filtrate was washed with 5% aqueous sodium hydroxide solution (2X50ml), 2% brine solution (75ml) and dried over anhyd.sodium sulfate. After distilled off the solvent, a dark red semi solid was obtained. The product was purified by column chromatography using benzene as eluent. The product was formed in 93.74% yield (6.19g) as red semi-solid. IR(KBr):1741 cm^{-1} (C=O of ester), 1686(C=O), 1596(aromatic C=C); ^{1}HNMR($CDCl_3$):4.20δ(q,2H, J=4Hz,COO$\underline{CH_2}CH_3$),1.28(t, 3H,J=4Hz;COOCH$_2\underline{CH_3}$), 1.96-2.72(m,9H, C_1-H,C_2-H, C_3-H &-S-CH_3, -CH_3),6.74-7.17(m,4H,Ar-H), 7.46-7.83(m,4H, Ar-H). Found: C, 71.15; H, 6.24, $C_{21}H_{22}O_3S$, required: C, 71.16; H, 6.26%.

Ethyl 2-(4-fluorophenyl)-3-(4-(methylthio)benzoyl)cyclopropanecarboxylate 14b: was obtained as a yellow colored solid. The product was obtained in 91% yield (5.99g), m.p.65-68°c. IR(KBr):1738 cm^{-1} (C=O of ester), 1678(C=O), 1595(C=C of aromatic); ^{1}H NMR($CDCl_3$):4.20δ(q,2H,J=4Hz,-COO$\underline{CH_2}CH_3$),1.30δ(t,3H,J=4Hz,-COOCH$_2\underline{CH_3}$), 1.95-2.74δ(m,6H, C_1-H,C_2-H &C_3-H, S-CH_3), 7.47-7.86(m,4H, Ar-H), 7.16-7.27(m, 4H,Ar-H), 7.46-7.87(m, 4H, Ar-H). Found: C, 67.01; H,5.33, $C_{20}H_{19}FO_3S$, required: C, 67.01; H, 5.32%.

Ethyl formate compound with (2-cyclohexyl-3-methylcyclopropyl)(4-(methylthio)phenyl)methanone 14c: was obtained as a orange coloured semisolid. The product was formed in 90.8% yield (6.39g), IR(KBr): 1740cm^{-1}(C=O of ester), 1683(C=O), 1597(C=C of aromatic); ^{1}H NMR($CDCl_3$): 4.21(q,2H,J=4Hz,-COO$\underline{CH_2}CH_3$), 1.27(t,3H, J=4Hz,-COOCH$_2\underline{CH_3}$), 1.98-2.72(m,6H,C_1-H,C_2-H,C_3-H & -S-CH_3), 7.46-7.86(m,4H,Ar-H), 7.13-7.27(m,4H,Ar-H).Found:C,69.55; H,8.23, $C_{20}H_{30}O_3S$, required: C,69.57; H,8.34%.

(1R,2R)-ethyl7-(methylthio)-4-oxo-1-p-tolyl-1, 2,3,4-tetrahydronaphthalene-2-carboxylate7:

A solution of cyclopropyl keto ester **14a** (5g, 0.0141mole) in dry dichloromethane (70ml) was added drop wise to a stirred solution of anhyd. stannic chloride (3.674g,0.0141mole)and acetic anhydride(2.87g, 0.0282mole) in dichloromethane (70ml) for an hr. at 0°C and further stirred for 6hr. After treating the reaction mixture with 5N HCl (50ml) solution, the organic layer was washed with 10% NaOH solution (2X50ml) and finally with water. The product was purified by column chromatography using benzene as eluent to give a brown solid in 82% yield (4.1g), IR(KBr):1744 cm^{-1} (C=O of ester), 1703(C=O), 1582(C=C of aromatic),^{1}H NMR ($CDCl_3$):3.92-4.21δ(q, 2H, J=4Hz, -COO$\underline{CH_2}CH_3$), 0.93-1.26(t, 3H, J=4Hz, -COOCH$_2\underline{CH_3}$), 2.55(s,3H,S-CH_3), 2.17(d,1H,J=3Hz,C_4-H),2.25-2.42(dd,2H,C_2-H),3.43-3.68(q,1H,J=3Hz,C_3-H), 6.97-7.19(m,5H, Ar-H),7.30-7.83(m,2H,C_5-H,C_8-H),7.28(d,1H,J=3Hz,C_7-H). Found: C, 77.15; H, 6.25, $C_{21}H_{22}O_3S$ required: C, 71.16; H, 6.26%. Mass spectra: (m/z, % abundance); m/z: 354.13 (100.0%), 355.13 (23.6%), 356.12 (4.5%), 356.14 (2.5%), 357.13 (1.0%).

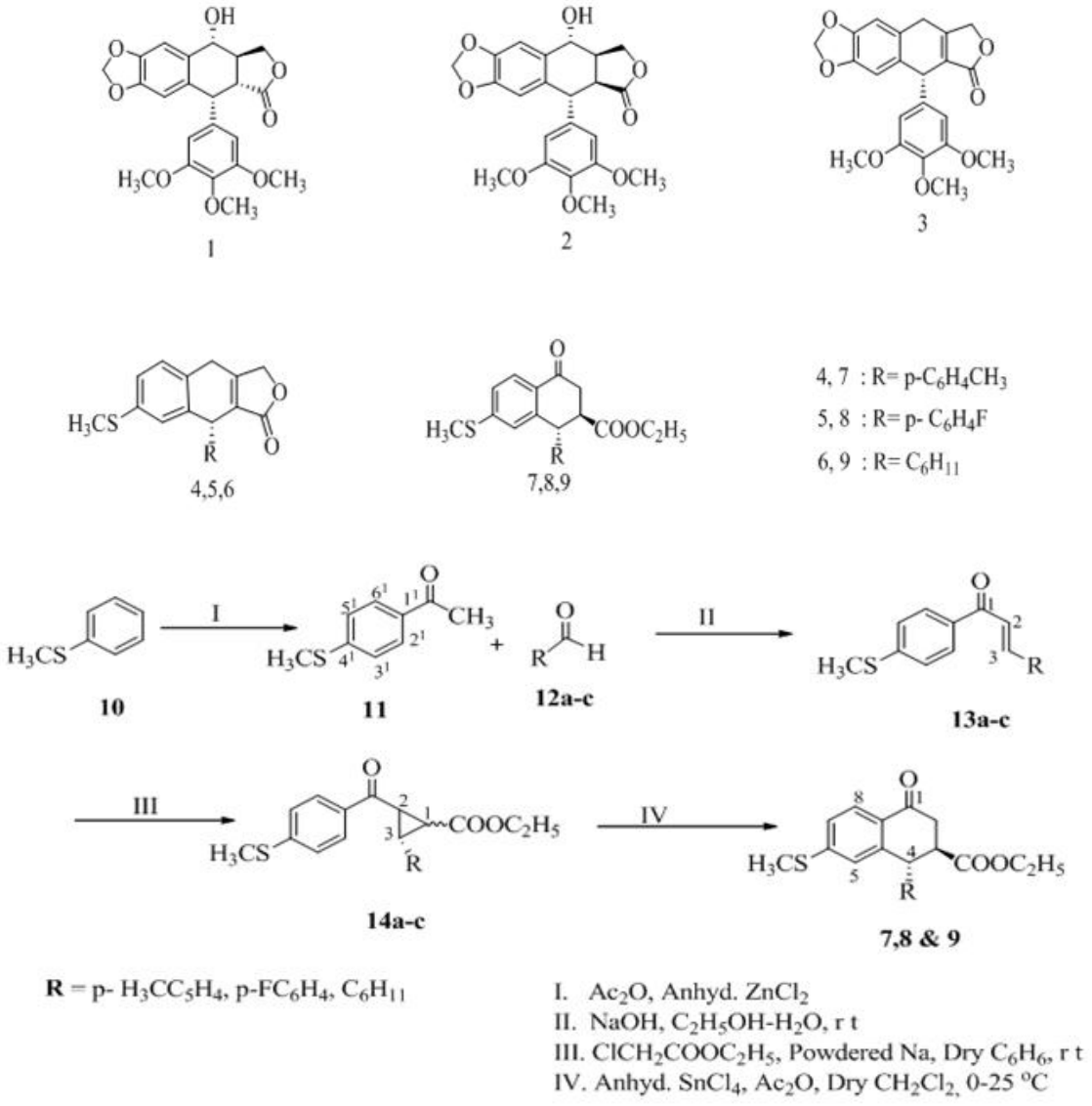
(2R)-ethyl1-(4-fluorophenyl)-7-(methylthio)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylate 8: was obtained as brown coloured semi-solid. The product was obtained in 88% yield(4.9g), IR(KBr):1737cm^{-1}(C=O of ester), 1701(C=O), 1598(C=C of aromatic); ^{1}H NMR($CDCl_3$); 7.18-7.27(m,4H,Ar-H),4.62(d,1H,J=4Hz,C_4-H),3.27-3.60(q,1H, J=4Hz, C_3-H), 2.71-3.03(dd,2H,C_2-H), 2.55(s,3H,-SCH_3), 7.20-7.81(m,3H,C_5-H,C_7-H &C_8-H),0.98-1.30-(t,3H,J=4Hz,-COOCH$_2\underline{CH_3}$),4.10-4.32 (q,2H, J=4Hz, -COO$\underline{CH_2}CH_3$). Found: C, 67.01; H, 5.35, $C_{20}H_{19}FO_3S$, required: C, 67.02; H, 5.34; Mass spectra: m/z: 358.10 (100.0%), 359.11 (22.0%), 360.10 (4.5%), 360.11 (3.1%) .

(2R)-ethyl1-cyclohexyl-7-(methylthio)-4-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylate 9: was obtained as orange coloured semi solid. The product was obtained in 78% yield (3.9g). IR(KBr):1745 cm^{-1} (C=O of ester), 1696(C=O), 1597(C=C of aromatic); ^{1}H NMR($CDCl_3$): 2.53δ(s,3H,-SCH_3), 0.93-1.67(t,3H,J=4Hz, -COOCH$_2\underline{CH_3}$), 4.13-4.47(q,2H,J=4Hz,COO$\underline{CH_2}CH_3$),7.51-7.92(m, 4H,Ar-H), 6.74-7.23(m,3H,Ar-H),2.23-2.37(dd,2H,C_2-H),2.16(d,1H, J=3Hz,C_4-H), 3.36-3.67(q,1H, J=3Hz, C_3-H).Found:C,69.32; H,7.54; $C_{20}H_{26}O_3S$, required: C, 69.33; H, 7.56%, Mass spectra: m/z: 346.16 (100.0%), 347.16 (22.5%), 348.16 (5.3%), 348.17 (2.3%), 349.16 (1.0%).

Antifungal Activity

The *in vitro* antifungal activity was carried out against seven fungi by Agar well diffusion method[13]. Compounds (7, 8 and 9) have been tested for their antifungal activity against *Trichoderma harzianum, Aspergillus niger, Colletotrichum capsici, Aspergillus tamari, Aspergillus flavus, Alternaria solani and Penicillium oxalicum*, at five

concentrations of 20, 40, 60, 80 and 100 µg/ml in chloroform. Potato dextrose agar plates were seeded with the test fungi in to the surface of the medium uniformly spread using sterile cotton bud. About 50 µl (500 µg/well) of each compound was loaded into the well (6mm diameter) made by sterile cork borer, then the plates were covered with par film incubated at 27 °C for seven days. Nystatin and sterile distilled water (SDW) were used as positive and negative control all over the experiment of 50 µg concentration.



Scheme-1

Table 1: compound number **7** dissolved in chloroform in different concentration:

Conc. In µg/ml	Fungi (zone of inhibition in mm)																				
	A			B			C			D			E			F			G		
	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct
20	14	12	10	22	14	13	17	16	10	15	14	10	17	13	11	16	14	09	27	26	17
40	14	11	10	22	14	13	17	17	10	15	13	10	17	15	11	16	14	09	27	27	17
60	14	13	10	22	13	13	17	14	10	15	12	10	17	15	11	16	12	09	27	25	17
80	14	13	10	22	15	13	17	14	10	15	12	10	17	12	11	16	12	09	27	23	17
100	14	11	10	22	16	13	17	15	10	15	11	10	17	14	11	16	13	09	27	21	17

Table 2: compound number **8** dissolved in chloroform in different concentration:

Conc. In µg/ml	Fungi (zone of inhibition in mm)																				
	A			B			C			D			E			F			G		
	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct
20	16	14	11	21	16	10	16	14	08	18	17	10	17	11	10	22	20	10	25	22	12
40	16	15	11	21	15	10	16	14	08	18	12	10	17	11	10	22	15	10	25	20	12
60	16	15	11	21	14	10	16	13	08	18	17	10	17	11	10	22	16	10	25	17	12
80	16	14	11	21	14	10	16	11	08	18	16	10	17	13	10	22	16	10	25	17	12
100	16	13	11	21	13	10	16	11	08	18	11	10	17	14	10	22	18	10	25	15	12

Table 3: compound number **9** dissolved in chloroform in different concentration:

Conc. In µg/ml	Fungi (zone of inhibition in mm)																				
	A			B			C			D			E			F			G		
	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct	N	S	Ct
20	12	15	10	20	14	11	19	17	10	17	11	11	17	12	10	17	15	06	21	20	13
40	12	13	10	20	14	11	19	20	10	17	15	11	17	12	10	17	12	06	21	19	13
60	12	14	10	20	13	11	19	18	10	17	11	11	17	11	10	17	12	06	21	19	13
80	12	13	10	20	12	11	19	18	10	17	13	11	17	13	10	17	11	06	21	15	13
100	12	12	10	20	16	11	19	16	10	17	12	11	17	11	10	17	13	06	21	16	13

Note: - A: Trichoderma harzianum; **B:** Aspergillus niger; **C:** Collectotrichum capsici;
D: Aspergillus tammari; **E:** Aspergillus flavus; **F:** Alternria solani ; **G:** Penicillium oxalicum;
N: Nistatin, positive control; **S:** Zone of inhibition of compound; **Ct:** Distilled water, negative Control

Evaluation of Antifungal activity

The antifungal activity of tetralone ester intermediates was tested against seven fungal species at the five concentration levels. The inhibition zone diameter was measured and tabulated in table 1, 2 and 3. The result showed the compounds possessed some inhibitory activities against one or more test organisms.

In compound number **7** the inhibitory activity was maximum against *Penicillium oxalicum* which showed 27mm at 40 µl concentration followed by 26mm at 20 µl when compared to nystatin showing 27mm at both 20 µl, 40 µl and control which showed least inhibition of 17mm at both concentrations compared to other fungi. Least activity was observed against the fungi *Tricoderma harzianum* which showed 13mm at both 60 and 80 µl concentrations when compared to the positive control showing 14mm and negative control having 10mm inhibition.

In case of compounds **8** and **9** showed considerable inhibition against *Penicillium oxalicum* when compared to all other fungi.

RESULTS AND DISCUSSION

In this context, we have chosen chalcone route with some changes in the experimental procedure and reagents to synthesise tetralone ester intermediates **7**, **8**, and **9** for podophyllotoxin analogues **4**, **5** and **6** (**scheme-1**). The starting material thioanisole **10** was prepared in high yield by the reaction of thiophenol with dimethyl sulphate in presence of sodium hydroxide in water. 4^1-Methylthio-acetophenone **11** was prepared in high yield by the Friedel-Crafts acylation reaction of thioanisole with acetic anhydride in presence of zinc chloride. Chalcones **13a-c** were prepared in excellent yields by Claisen condensation reaction of 4^1-methylthio acetophenone **11** with benzaldehydes **12a-c** in the presence of sodium hydroxide in water-ethanol mixture[14]. Cyclopropyl keto esters **14a-c** was prepared in good yields by the reaction of chalcones **13a-c** with ethyl chloro acetate in the presence of powdered sodium in dry benzene.

Tetralone ester intermediates 7, 8 and 9 were prepared in good yields by the intramolecular Friedel-Crafts alkylation reaction of cyclopropyl keto esters 14a-c in the presence of anh. Stannic chloride and acetic anhydride in dry dichloromethane[15]. The structure of tetralone esters were based on IR, ^{1}H NMR, mass spectra and elemental analysis data. The synthesized compounds were screened for antifungal activity. The results are summarized in Table 1, Table 2 and Table 3.

CONCLUSION

We have successfully synthesized new analogues of tetralone esters. All compounds were characterized by standard spectroscopic techniques. The evaluation of the antifungal activity of all the new compounds was carried out against fungi and proved significant to moderate activity.

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