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Preparation and in Vitro Cytotoxic Evaluation Studies of Ethyl-4-methyl-6-(7-methoxy-2-oxo-2H-chromen-8-yl)-hexa-2,4-dien-1-oate, an Analogue of Osthol

Shahnawaz Shaheen, Zubaid ul Khazir, Javid A. Banday*

Department of Chemistry, National Institute of Technology Srinagar, Hazratbal, Srinagar, J&K, India.

ABSTRACT

Coumarin, Osthol was isolated from the Plant Prungos pabularia. Osthol was then subjected to the chemical modification and the corresponding aldehyde and esters were prepared from it. The ester was then evaluated for the anticancer activity against four cancer cell lines. The ester derivative showed better anti cancer activity than the parent compound Osthol.

Keywords: Aldehyde, Anticancer Activity, Ester, Osthol, Prungos Pabularia.

I INTRODUCTION

Coumarins and their derivatives are reported to display numerous biological and pharmacological activities[1]. For example (+)-Heraclenin, a naturally occurring furanocoumarin epoxide, is known to exhibit a broad spectrum of biological activities, like cytotoxic[2], antiplatelet[3], anti-coagulant[4], anti-inflammatory[5,6] as well as mild phototoxic and photomutagenic activities[7]. It has been shown to significantly induce apoptosis in Jurkat leukaemia cells[8] Spectrum of activities of several similar furanocoumarins and their glucosides has also been reported in literature[9].

II METHODOLOGY

2.1 General

IR spectra were recorded on Perkin-Elmer Paragon-1000 spectrophotometer Esquire 3000 spectrometer. 1H spectra were recorded at 400 MHz and 13C NMR at 100 MHz on 500 Bruker Avanc instruement using TMS as internal standard and CDCl3 as the solvent. High resolution mass spectra were recorded on Agilent (QTOF hybrid). Column chromatography was carried out on Merk silica gel (60-120 mesh and 100-200 mesh). Aluminium sheets, precoated with silica gel 60 F254 (20x20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by cerric sulfate as spraying reagent

2.2 Plant Material

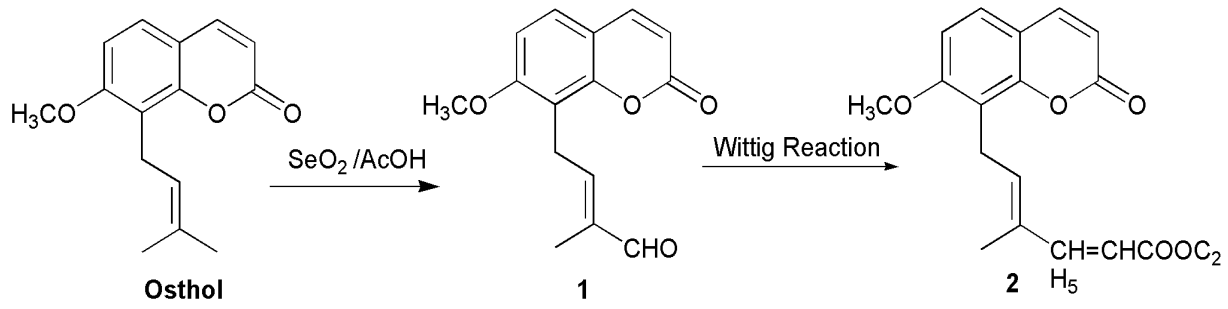
The plant *Prangos pabularia* was collected from Drass, Ladakh (J&K, India). The specimen was identified by Akhtar H. Malik, Curator, Centre for Biodiversity & Taxanomy, University of Kashmir (Voucher Specimen No. 1845 KASH Herbarium, University of Kashmir, 08.05.2013, Drass).

2.3 Extraction and Isolation

The air dried, finely powdered root material (2Kg) was extracted for 72 hours sequentially with petroleum ether (60-80^{o}C), ethyl acetate and methanol in a soxhlet apparatus to afford the respective extracts, which were concentrated under reduced pressure and were coded as PPP, PPE and PPM, respectively. Osthol was isolated from petroleum ether extract by column chromatography using silica gel as adsorbent and petroleum ether-chloroform (4:1) as eluent. Its structure was elucidated on the basis of extensive spectral techniques like MS, IR, UV, 1H NMR and 13C NMR.

2.4. Procedure for synthesis of 2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al **(1)** and Ethyl-4-methyl-6-(7-methoxy-2-oxo-2H-chromen-8-yl)-hexa-2,4-dien-1-oate **(2).**

Compound **1** (Aldehyde) was prepared by adding selenium dioxide (1 eq.) to a solution of osthol (1 eq.) dissolved in glacial acetic acid (7ml) and stirred for about 3 hrs. On completion of the reaction (monitored by TLC), the contents were poured into crushed ice and extracted with dichloromethane (50 ml), dried over sodium sulfate and concentrated on rotary-vacuum evaporator to give crude product, which on silica gel column chromatography, using Pet.ether-ethyl acetate as an eluent, yielded pure aldehyde **1** in 45% yield.



Osthol 1 2

Compound **2** (Ester) was prepared by adding dry NaH (in excess) to Wittig reagent dissolved in dry ether at 0^{o}C under nitrogen atmosphere, and the contents stirred for 30 minutes followed by addition of an ethereal solution of compound **1** (aldehyde, 1 eq.) and the contents stirred for 2 hrs. The reaction mixture after the completion of the reaction worked up by dilution with ethyl acetate to quench excess of NaH, followed by careful addition of water,

extraction with ethyl acetate (50 ml), dried over sodium sulfate and concentrated to give crude product **2**. Subsequent purification of the product by silica gel column chromatography using Pet. ether-ethyl acetate as the eluent afforded pure ester **2** in 87% yield.

2.5. Spectral analysis of Ethyl-4-methyl-6-(7-methoxy-2-oxo-2H-chromen-8-yl)-hexa-2,4-dien-1-oate (2).

The product after isolation and purification (Compound 2), in its IR spectrum, displayed a prominent band at 1687 cm^{-1}due to unsaturated ester, in addition to the band at 1723 cm^{-1}due to lactone carbonyl group. This was further supported by proton spectrum, wherein a down field signal at δ 1.96 (singlet) for only three protons, a triplet at δ 1.23 for three protons (*J*= 7.19 Hz, -$OCH_2\underline{CH_3}$) and a quartet for two protons (*J*= 7.11 Hz, -$O\underline{CH_2}$-CH_3), were observed.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (3H, t, *J*= 7.19 Hz, -$OCH_2\underline{CH_3}$),1.96 (3H, s, $\underline{CH_3}$-C=), 3.66 (2H, d, *J*= 7.50 Hz, Ar-CH_2-$\underline{CH}$=), 3.89 (3H, s, Ar-OMe), 4.08 (2H, q, *J*= 7.11 Hz, -$O\underline{CH_2}$-CH_3), 5.75 (1H, d, *J*= 15.33 Hz, -CH=$\underline{CH}$-CO), 5.88 (1H, t, *J*= 7.48 Hz, -$\underline{CH}$=CH_2-Ar), 6.77 (1H, d, *J*= 8.6 Hz, -CH=$\underline{CH}$-CO), 7.18 (1H, d, *J*= 8.19 Hz, Ar-H), 7.70 (1H, d, *J*= 15.86 Hz, -$\underline{CH}$=CH-COO-), 7.26 (1H, d, *J*= 8.7 Hz, -$\underline{CH}$=CH-COO-) 6.19 (1H, d, *J*= 9.46 Hz, -CH=$\underline{CH}$-CO).

The structure was further confirmed by ^{13}C NMR,mass spectra and IR spectra.

^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.74, 160.97, 160.49, 153.25, 143.74, 127.50, 127.50, 113.16, 113.09, 112.95, 112.05, 107.27, 64.40, 56.13, 40.91, 34.70, 31.93, 29.70, 22.69, 18.43.

EIMS *m/z*: 351.11(M^+ + Na), 351, 327(M+ - 1), 283(M+-OEt), 189, 159, 131.

IR (KBr) $\nu_{max}$$cm^{-1}$: 2923, 2851, 1723, 1607, 1497, 1463, 1402, 1282, 1252, 1163, 1117, 1034, 833, 774.

III BIO-EVALUATION STUDY OF COMPOUND 2

The compound **2** along with Osthol, was evaluated for its anticancer activity against breast (MCF-7), ovary (IGR-OV-1), lung (A-549) and prostate (PC-3) human cancer cell lines at different concentrations, paclitaxel as the standard (Table-1).

Compound **2** exhibited excellent activity at 60 μM concentration displaying inhibition of 83%, 74%, 80% and 58% against four cancer cell lines namely lung (A-549), breast (MCF-7), ovary(IGR-OV-1) and prostate (PC-3) cell lines, respectively. At 30 μM concentration, an inhibition of 58% against breast (A-549) cell line and 50% inhibition against breast (MCF-7) was observed. It is pertinent to mention that in the parent molecule, Osthol, the inhibition observed against all the cancer cell lines was comparatively very low at all the four concentrations taken in the present study.

Table-5.5. Anticancer activity data of osthol analogues

Tissue		Colon	Lung	Leukemia	Breast	Ovary	Prostate
Cell Line Type		Colo-205	A-549	THP-1	MCF-7	IGR-OV-1	PC-3
Compound	Conc.(µM)	% Growth Inhibition					
2	60	-	**83**	-	**74**	**80**	**58**
	30	-	**58**	-	**50**	38	08
	15	-	21	-	35	10	0
	7.5	-	0	-	05	02	0
Osthol	60	-	38	-	47	**60**	16
	30	-	20	-	30	18	10
	15	-	07	-	23	12	0
	7.5	-	0	-	07	12	0
Paclitaxel	1	-	66	-	-	65	-

Keeping in view the significant activity profile of compound **2**, it is of interest to carry forward these cytotoxic compounds for mechanistic and *in vivo* studies to establish whether these agents act through apoptosis or by necrosis, as well as to further modulate the structural frame work of these molecules for their higher potency and better therapeutic effect.

IV CONCLUSION

Osthol and its ester derivative were evaluated for biologically activity and it was found that the ester derivative has better anticancer activity than the parent compound. The researchers are attracted to fine tune the properties of the natural products and evaluating their structural activity relationship. We have attempted to fine tune the properties of osthole and further work needs to be done.

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