International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 5, Issue 1
January 2018

Preparation and in Vitro Cytotoxic Evaluation Studies of 2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al, an Analogue of Osthol

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

Abstract

Anticancer activity of 2- Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al, an analogue of osthol was performed on various human cancer cell lines viz; evaluated for in vitro anticancer activity against Colon (Colo-205), lung (A549) , leukemia (THP1) and breast (MCF-7) human cancer cell lines at four different micro-molar concentrations (7.5 µM, 15 µM, 30 µM, and 60 µM).

Key words: Prangos pabularia, Osthol, Aldehyde, Modifications, Anticancer activity.

1. Introduction

Cancer remains one of the most common and fatal disease responsible for 2-3% of deaths recorded worldwide annually. The effectiveness of many anticancer drugs is limited by development of drug resistance, which may be intrinsic or acquired during the course of treatment and found to be responsible for treatment failure in more than 90% of patients with metastatic disease.

Medicinal plants have proved a promising source of novel chemotherapeutic agents including cancer and interestingly about 60% of anticancer drugs used nowadays are obtained from natural sources [1]. Further, the chemical modification of plant derived natural product/s has resulted in better anticancer activity well exemplified by topotecan and irinotecan, the synthetic derivatives of camptothecin [2]. Therefore, to achieve better therapeutic impact, i.e., lower sensitivity and higher efficacy, chemical modification of natural product/s apparently looks an interesting proposition.

Coumarins (and their derivatives) represent an important class of compounds reported to display numerous biological and pharmacological activities [3]. For example (+)-Heraclenin, a naturally occurring furanocoumarin epoxide, is known to exhibit a broad spectrum of biological activities, like cytotoxic [4], antiplatelet [5], anti-coagulant [6], anti-inflammatory [7,8] as well as mild phototoxic and photomutagenic activities [9]. It has been shown to significantly induce apoptosis in Jurkat leukaemia cells [10]. Spectrums of activities of several similar furanocoumarins and their glucosides have also been reported in literature [11]. In present study, we report the preparation of 27 analogs of osthol (isolated from the root part of the plant *Prangos pabularia*), incorporating modifications in both the rings of the molecule as well as in the prenyl side chain. The duly chemically characterized molecules and osthol were bio-evaluated against several human cancer cell lines

2. Results and Discussion

Compound **1** was obtained by the oxidation of osthol with selenium dioxide in acetic acid. In its IR spectrum, a prominent band at 1681 cm^{-1}, due to unsaturated aldehyde carbonyl was observed in addition to the band at 1728 cm^{-1}due to lactone carbonyl group. However, no twin peaks at 1385 & 1362 cm^{-1}, characteristic of

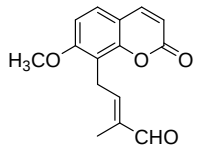
International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 5, Issue 1
January 2018

gemdimethyl, were observed. This was further supported by proton spectrum, wherein a down field signal at δ 1.95 (singlet) for only three protons, and signal for aldehydic proton at δ 9.2, were observed. The structure was further confirmed by ^{13}C NMR and mass spectrum.

Compound **1** was bio-evaluated against breast Colon (Colo-205), lung (A549) , leukemia (THP1) and breast (MCF-7) human cancer cell lines at four different micro-molar concentrations (7.5 µM, 15 µM, 30 µM, and 60 µM), taking mitomycin, adriamycin and paclitaxel as the standard.

The cells were treated with test compounds (dissolved in DMSO) at the above said concentrations and kept in serum media for 48h to observe anti-proliferative activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The results revealed significant sensitization of the cell lines by inhibiting their cell growth (Table 1).

Table1: Percentage Growth Inhibition at 7.5 µM, 15 µM, 30 µM, 60 µM and IC_{50} values of compound 1 against Colo-205, A549, THP-1 and MCF-7 cell lines with MTT assay. BEZ-235(0.01µM), Paclitaxel (1µM) and Adriamycin (1µM) were used as positive controls.

Tissue			Colon	Lung	Leukemia	Breast
Cell Type			Colo-205	A549	THP-1	MCF-7
Entry	Conc.(µM)		Percentage Growth Inhibition			
 1	60		48±3	51±1	41±2	25±3.2
	30		29±2.4	37±2	26±3.3	21±2.8
	15		19±2	35±3.2	13±1.4	19±1.2
	7.5		12±1.6	28±4.4	9±1	13±1.3
IC_{50}			>60µM	>60µM	>60µM	>60µM
	BEZ-235	0.01	35±3	32±4	31±5	47±3
	Paclitaxel	1	-	71±4	-	-
	Adriamycin	1	-	-	-	82±5

The activity profile of the modification product (**1**) reveals its significant activity against Colo-205 and A-549 at different concentrations. The results warrant further study in the light of developing new anticancer drugs of better therapeutic impact by chemical modifications of the parent molecules.

3. Experimental

3.1. *General*

IR spectra were recorded on Perkin-Elmer Paragon-1000 spectrophotometer Esquire 3000 spectrometer. ^{1}H spectra were recorded at 400 MHz and ^{13}C NMR at 100 MHz on 500 Bruker Avanc instruement using TMS as internal standard and $CDCl_3$ 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 F_{254} (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.

3.2. *Plant Material*

The root parts of *Prangos pabularia* (10 Kg) were collected from Drass, Ladakh (J&K, India) in July 2008. The specimen was identified by Akhtar H. Malik, Curator, Centre for Biodiversity & Taxanomy, University of Kashmir (Specimen deposited under accession No. 33214 and Collection No. 1203- Javid, Kash).

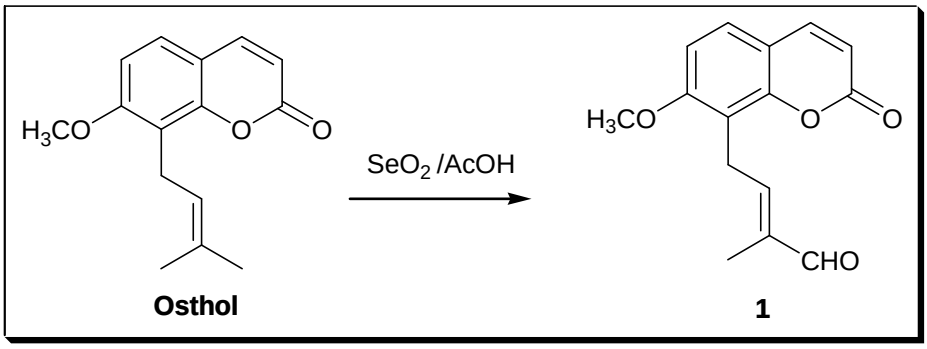
International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 5, Issue 1
January 2018

3.3. *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, ^{1}H NMR and ^{13}C NMR.

3.4. General procedure for synthesis of 2-Methyl-4-(7-methoxy-2-oxo-2H- chromen-8-yl)-but-2-en-1-al (1)

Compound **1** 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 rotavapor to give crude product, which on silica gel column chromatography, using Pet.ether-ethyl acetate as the eluent, yielded pure aldehyde (**1**) in 70% yield.



2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al (1)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, s, CH_3-C=), 3.94 (3H, s, Ar-OMe), 3.89 (2H,d, *J*=7.69 Hz Ar-CH_2-C=), 6.28 (1H, d, *J*= 9.46 Hz, -CH=CH-CO), 6.53 (1H,t, *J*= 7.34 Hz, Ar-CH_2-CH=), 7.39 (1H, d, *J*= 8.62 Hz, Ar-H), 6.88 (1H, d, *J*= 8.6 Hz, Ar-H), 7.66 (1H, d, *J*= 9.5 Hz, -CH=CH-CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 195.4, 160.79, 160.30, 153.0, 150.74, 143.64, 139.81, 127.38, 114.27, 113.33, 113.10, 107.4, 50.19, 22.8, 9.27.

EIMS *m/z*: 258.2482 [M^+]. IR (KBr) ν_{max}cm^{-1} : 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510

Acknowledgment

Thanks are due to the Director NIT Srinagar for providing all necessary facilities available to carry out the present work.

References

1. Sakpakdeejaroen, I., Itharat, A. 2009. *J Health Res.*, ***23***, 71-76.
2. Daniels, A. L., Slambrouck, S. V., Lee, R. K., Arguello, T. S., Browning, J., Pullin, M. J., Kornienko, A., Steelant, W. F. A. 2006. *in vitro. Onco. Rep.*, ***15***, 1327-1331.
3. Hoult, J. R.; Payá, M. *Gen. Pharmacol.* **1996**, *27*, 713.
4. Setzer, W. N.; *et al*. *Planta Med.* **2000**, *66*, 493.

5. Shenghong, Li.; Xuemei, Sun.; Handong Lin.; Zhongwen. *Farming Zhuanli Shenqing Gongkai Shuomingshu*, **2005**, *15*, 8.
6. Mishra, K. C.; Sharma, R. C.; Sharma, Y. N.; Arora, R. B. *J. Physiol. Pharmacol.* **1969**, *13*, 153.
7. Garcia, Argaez, A. N.; Ramirez, A.; Teresa, O.; Delgado, H. P.; Velazquez, G.; Martinez, Vazquez, M. *Planta Med.* **2000**, *66*, 279.
8. Bal-Tembe, Swati, J.; Deepak, D.; Lakdawala, A. D. *Indian J. Chem. B.* **1996**, *35*, 518.
9. Schimmer, O.; Abel, G. *Mutat. Res.* 1986, *169*, 47.
10. Appendino, G. *et al*. *J. Nat.Prod*. **2004**, *67*, 532.
11.Tada, Y.; Shikishima, Y.; Takaishi, Y.; Shibata, H.; Higuti, T.; Honda, G.; Ito, M.; Takeda, Y.; Kodzhimatov, O.; Ashurmetov, O.; Ohmoto, Y. *Phytochemistry*, **2002**, *59*, 649.